Technische Universität München

TECHNISCHE UNIVERSITÄT MÜNCHEN

TUM School of Life Sciences

Milk protein powder solubility and rennet gelling functionality for cheese production

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Vollständiger Abdruck der von der TUM School of Life Sciences der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktorin der Ingenieurwissenschaften (Dr.-Ing.)

genehmigten Dissertation.

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Prüfende der Dissertation:	1. Prof. Dr.-Ing. Ulrich Kulozik
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	3. Prof. Dr.-Ing. Heiko Briesen

Die Dissertation wurde am 02.09.2021 bei der Technischen Universität München eingereicht und durch die TUM School of Life Sciences am 27.04.2022 angenommen.

Egal was kommt, es wird gut, sowieso
Immer geht 'ne neue Tür auf, irgendwo
Auch wenn's grad nicht so läuft, wie gewohnt
Egal, es wird gut, sowieso.

- Mark Forster

Danksagung

Die vorliegende Arbeit entstand im Rahmen meiner Tätigkeit am Lehrstuhl für Lebensmittel- und Bio-Prozesstechnik im Zeitraum von 2017 bis 2021. Sie wurde von Prof. Dr.-Ing. Ulrich Kulozik betreut, dem ich an dieser Stelle meinen besonderen Dank aussprechen möchte. Ich schätze seine wissenschaftliche, finanzielle und persönliche Unterstützung sowie das hohe Maß an akademischer Freiheit, das er mir gewährte.

Ein herzliches Dankeschön an all meine Kolleginnen und Kollegen, die dazu beitrugen, dass das Institut ein ganz besonderer Arbeitsplatz war. Insbesondere möchte ich mich bei meinen Bürokolleg*innen Anne-Martha Schwarz, Alexander Herzum und Roland Schopf für eine entspannte und angenehme Atmosphäre bedanken.

Ich möchte mich bei meinen Student*innen Lorenz Thurin, Eva-Maria Schmid, Than Mai Nguyen, Daniela Panoch und Sonja Keienburg bedanken, die zu dieser Arbeit beigetragen haben.

Ein besonderer Dank geht an meine Kolleg*innen Roland Schopf, Ingrun Kieferle, Simon Schiffer, Mine Özcelik, Jannika Dombrowski, Martin Hartinger und Michael Reitmeier für Ihre Unterstützung und wertvollen Gespräche und Diskussionen.

Des Weiteren danke ich Günther Unterbuchberger und Andreas Matyssek für ihre tatkräftige Unterstützung bei den Technikumsversuchen; Claudia Hengst, Hermine Roßgoderer, Heidi Wohlschläger, Martin Hilz, Annette Brümmer-Rolf und Christine Haas für die Analytik meiner Proben; dem Werkstattpersonal Franz Fraunhofer, Christian Ederer und Erich Schneider, die stets bei technischen Fragen und Problemen zur Seite standen.

Ein großer Dank geht außerdem an meinen Projektpartner Søren Christian Lillevang von Arla Foods amba.

Vielen Dank für alles!

Content

1. General introduction

Milk is dried in huge amounts and reconstituted prior to the manufacture of various dairy and other food products. Milk powders exist in various forms differing in protein composition and content of low molecular weight constituents. High protein powders, such as milk protein concentrate (MPC) or micellar casein powders (MC) can be used, for instance, to increase the total protein content or to vary the casein/whey protein ratio of cheese milk to enhance the yield (Anema et al., 2006; Guinee et al., 2006; Singh, 2007). Using milk powders, the protein content can be standardized flexibly, and seasonal fluctuations of the milk composition can be compensated (Guinee et al., 2006). However, the redispersion of high protein powders can be challenging in terms of solubility (Baldwin and Truong, 2007), which is often neglected in studies assuming that rehydration overnight would ensure full powder dissolution. An insufficient powder rehydration may affect the rheological properties and the rennet gelation behavior of protein powder-enriched milk. Powders with a poor solubility may force the end-user to modify existing unit operations or product formulations adapting them to the powder in order to possibly achieve faster powder rehydration (Crowley et al., 2015).

For traditional cheese manufacture, the casein concentration is decisive since the whey proteins are not part of the rennet gel network and drain with the sweet whey. Considering that 70% of the starting amount of milk is converted to sweet whey during cheese manufacture, a huge amount of valuable whey proteins is not incorporated in the final product. The sweet whey is commonly purified to obtain the isolated whey proteins. However, in some parts of the world with lacking infrastructure for whey processing, the whey must be discarded, creating a potential environmental burden. To avoid losses of valuable whey proteins, heat treatment could be applied up to a certain degree to denature and structurally integrate the whey proteins in the cheese curd without impairing the rennetability.

The concept of this thesis, in brief, therefore, was as follows: Powder rehydration conditions will be investigated to define a standard process for powder redispersion which leads to full solubilization of even poorly soluble powders. The focus will be on low shear and high shear units such as rotor/stator mixing systems like in shear pumps or high pressure homogenizers. These high shear units run continuously and are commonly implemented in dairies and can be used for powder rehydration. A powder counts as fully solubilized when the particle size distribution corresponds to that of fresh skim milk. So the question was, under which rehydration conditions specific powder types fully solubilize.

In addition to the investigation of the functional properties of industrial milk powders, a process for functionalized powders for cheese production will be developed. The feed concentrate plays an important role here. Changing the functional properties by concentrating, diafiltrating, heating, or renneting affect its processability inevitably. Therefore, it is essential to understand how the milk proteins are affected by these treatments, so that the process can be controlled in a targeted manner.

For a better understanding of the results presented in the form of publications, the overall state of knowledge and the basics of the methodologies applied as well as the required details of the chemico-physical aspects of milk components—especially the milk proteins—will be presented in more detail than possible in comprehensive journal articles.

1.1. Milk proteins

For the production of dairy powders, milk is most often processed in such way, that the physical state and/or composition in terms of their main components (proteins, lactose, milk fat, milk salts, and water) change. `Skim milk´, which is also used for the experiments presented in this work, is milk after centrifugal fat removal. Table 1.1 gives the overall composition of unconcentrated, bovine skim milk.

Table 1.1 Composition of milk (Töpel, 2016).

Component	Concentration, g kg^{-1}	Component	Concentration, g kg^{-1}
Water	860 - 880	Lactose	46.7 - 48
Lipids	30 - 45	Salts	6.0 – 7.5
Proteins	32- 36		
Caseins	26 - 30	*Whey proteins*	6 – 6.2
α_{s1}-Casein	10.3 – 11.9	β-Lactoglobulin	3.1 – 3.5
α_{s2}-Casein	2.6 -3.2	α-Lactalbumin	1.2 – 1.3
β-Casein	9.9 – 11.9	BSA	0.4
κ-Casein	3.3 – 3.5	other	1.9-2.3

In the following, the structure of the main protein fractions, the caseins and the whey proteins, as well as their structural changes under selected processing treatments are presented, since these details are important as a base for a good comprehension of the methodology chosen and results presented.

1.1.1. Caseins

The caseins are the main components of the milk proteins, accounting for 78-82%, or 2.6-3.2% of total milk. The casein micelle consists of several protein fractions, namely α_{S1}, α_{S2}, β, and κ-casein (Schmidt, 1980).

The monomeric primary caseins share some common features, such as that they all consist of an un-branched polypeptide chain with molecular masses of 19,000-24,000 g mol^{-1}, which include hydrophilic and hydrophobic sequence regions. In addition, all fractions contain ester phosphate whose residues esterified with the serine's hydroxyl group. Due to this, these caseins tend to have an increased binding of calcium and magnesium, which increases with the number of phosphoseryl groups ($\alpha_{S1} > \alpha_{S2} > \beta > \kappa$). Furthermore, they have a high content of proline, which is evenly distributed throughout the molecule, disrupting the α-helix and β-sheet structures and thus, preventing the formation of a secondary structure (Töpel, 2016).

In milk, caseins are present in a complex micellar form (Horne, 2009) as shown in Figure 1.1. Since the 19th century, the casein micelle is considered as a colloidal particle with a stabilizing surface layer consisting of κ-casein (Dalgleish, 1998; Fox et al., 2015).

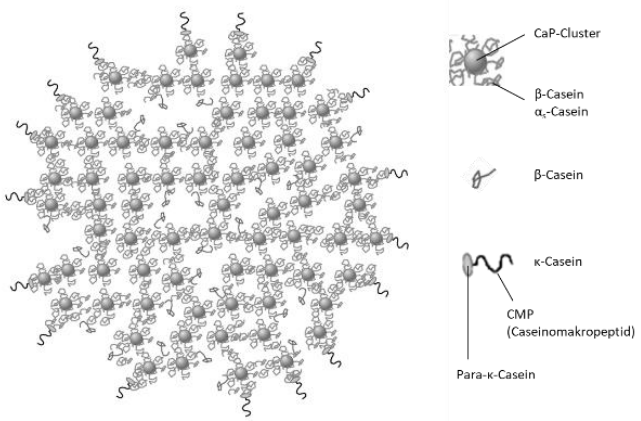


Figure 1.1 Schematic illustration of the casein micelle (Dalgleish and Corredig, 2012).

To describe some aspects of the behavior of casein micelle, it is simplistically assumed to be a stable sphere. However, it has already been demonstrated by numerous authors that it is a much more complex structure whose subtleties cannot be explained by this simplification (Dalgleish, 1998). Depending on their size, casein micelles consist of approximately 100-300,000 casein polypeptide chains. In the hydrated state, they have molecular masses of up to 1.3×10^9 g mol^{-1}. The mass ratio of α_{S1}, α_{S2}, β, and κ is 4 : 1 : 4 : 1.3 (Walstra, 1990). 94% of the micellar dry mass consists of proteins, whereas 6% consists of colloidal calcium phosphate.

The mineral fraction consists mainly of calcium and phosphate, as well as the minor components magnesium and citrate. The micelles have an average diameter of ~120 nm. One milliliter milk contains 10^{14}-10^{16} micelles, which have an average distance of about 240 nm from each other. The surface area is 5×10^{-4} cm^2 mL^{-1} (Fox et al., 2015). Cryo-transmission electron tomography revealed that here are water channels and inclusions in the micellar structure. Hence, they can bind a lot of water, about 3.5 kg per kg protein. Therefore, casein micelles account for 10% of the milk's volume, but only for 2.5% of the milk's weight (Dalgleish and Corredig, 2012; Jeurnink and de Kruif, 1993). At the natural pH of milk (6.7-6.8), casein micelles are stabilized by two factors: 1) Negatively charged groups on their surfaces (-15 to -20 mV) inducing mutual repulsion; 2) steric stabilization by κ-casein (Töpel, 2016).

The secondary structure of α_{S1}-, β-, and κ-casein consists of 20-29% α-helices, 32-34% β-sheet structures, 28-32% so-called loops, and 22-34% disordered structures (Farrell et al., 2001). Kumosinski et al. (1993) developed a model for the tertiary structure of the casein micelle using molecular level modeling techniques, predicted secondary structures and general empirical information on secondary structure by Raman spectroscopy. However, there is still too little experimentally obtained evidence of the actual folding of the polypeptide chains with which the model can be validated (Livney et al., 2004).

The quaternary structure has not been elucidated to date. Instead, models have been developed, e.g., the submicellar model (Schmidt, 1980; Slattery and Evard, 1973), the nanocluster model (Holt, 1998), and the dual-binding model (Horne, 1998).

A frequently discussed model for the structure of casein micelles is the submicelle model. For this, it was assumed that the casein micelle is nearly round, but does not have a smooth surface, and is composed of smaller units, the submicelles (Walstra, 1999). Slattery and Evard (1973) assumed that a spherical submicelle contains α-, β-, and κ-caseins that are aligned in a star shape around the center. The highly charged N-terminus of the β-casein, which contains the phosphate center, provides the amphiphilic character of the protein

(Swaisgood, 2003), favoring submicellar aggregation. The `hairy layer´ consists of a 5-10 nm thick layer of glycosylated and non-glycosylated κ-casein and small amounts of α_{s1}-, α_{s2}-, and β-casein. The hairs consist of the protruding molecular C-terminal chains of the κ-caseins. These are thought to stabilize the micelle and prevent further aggregation of the submicelles by steric stabilization and electrostatic repulsion (Fox et al., 2015; Walstra, 1999).

Since none of these models has been experimentally validated, the theories of steric stabilization of casein micelle still leave various questions unanswered, and many authors have started to explain the micellar structure with alternative stabilization mechanisms. Holt (1998) found that the phosphopeptide fraction of β-casein binds and stabilizes calcium phosphate aggregates. So-called `nanoclusters´ form in which the colloidal calcium phosphate is dispersed (de Kruif et al., 2012; Holt et al., 1998). de Kruif and Holt (2003) assumed that the hydrophobic polypeptide chains associate with other proteins (and these in turn with colloidal calcium phosphate) by hydrophobic interactions, hydrogen bonding, ionic bonding, weak electrostatic interactions, and other factors (excluding the strong calcium phosphate interactions). A more or less homogeneous protein matrix is formed (de Kruif et al., 2012). The colloid grows until it reaches its maximum size (~100 nm). The growth terminates if κ-casein binds since its hydrophilic C-terminus cannot bind to other micelles. Nanoclusters can consequently be described as a core consisting of calcium phosphate surrounded by a shell of phosphoproteins (Holt et al., 2009). A casein micelle contains about 800 such nanoclusters (Qi, 2007). Holt's model also describes the diffuse hairy surface. This hydrophobic, negatively charged, diffuse layer is responsible for the repulsion of the micelles from each other. If the hairs are removed enzymatically, for example by chymosin or destroyed by heat or acid, the micelles aggregate (Holt and Horne, 1996).

The dual-binding model states another type of cross-linking besides the nanoclusters. Hydrophobic interactions may be responsible for the self-association of caseins, as the formation of linkages is favored by a local excess of hydrophobic attractive forces over electrostatic repulsive forces. The integrity and stability of the micelle is thus maintained (Horne, 2009).

All presented models agree in the presence of κ-casein and its formation of a hairy surface. At normal milk pH, electrostatic repulsion dominates over hydrophobic attraction between casein micelles. However, this can shift due to pH or temperature changes or due to the addition of rennet, urea, or complexing agents (Horne, 2009). The changes that occur during processing or storage can be studied at different levels. At the macroscopic level, the technological properties are studied; at the mesoscale, the changing structure is investigated using chemical and physical measurement methods; and at the microscopic level, the quaternary structure of the proteins is studied. In order to understand the behavior and structural properties of casein micelles, it is mainly examined at the macroscopic level and at the mesoscale in terms of properties, since the tertiary and quaternary structure is still not clarified.

The casein micelles are specifically destabilized for many dairy products. For example, for yogurt manufacture, milk is acidified which leads to the dissolution auf the calcium phosphate and the collapse of the hairy layer. Consequently, a gel forms. Renneting is a key step for cheese manufacture, where the hairy layer of the casein micelles is enzymatically cleaved allowing a close approach of the casein micelles, the formation of calcium bridges, and consequently, coagulation. Since cheese making is an integral part of this work, the renneting process is described in more detail below.

The renneting process can be split in two phases, hydrolysis and aggregation. During hydrolysis, the rennet cleaves the κ-casein at the position Phe^{105}-Met^{106}. The hydrophobic N-terminus, which is the para-κ-casein, remains in the casein micelle, whereas, the hydrophilic C-terminus, the casein macropeptide (CMP) passes into the serum phase/sweet whey (Figure 1.2). The micelle loses its hydration shell through which the micelle volume and hence, the viscosity of the solution, decrease. A decrease in viscosity is observed above 85% hydrolysis degree (Tuinier and de Kruif, 2002). It is important to note that the hydrolysis step works at temperatures below 16 °C, whereas aggregation occurs only above 16 °C (Bansal et al., 2008, 2007), which can be used to steer the entire reaction and its effects by a two-step-process below and above 16 °C, respectively. The rennet is no longer involved in the second phase of the gelation process. Further to the lost steric stabilization, hydrophobic interactions dominate after hydrolysis. The casein micelles get closer and finally they aggregate via Ca^{2+}-bonds and form a gel (Dalgleish, 1979; Kethireddipalli et al., 2011; Sandra et al., 2007). This is mirrored in an increasing viscosity.

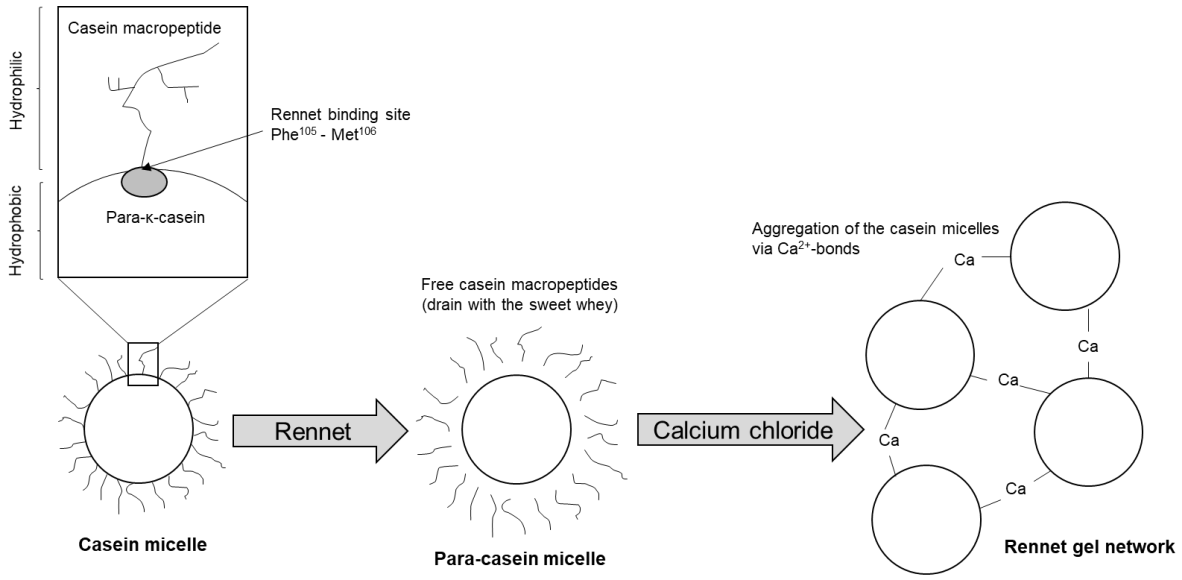


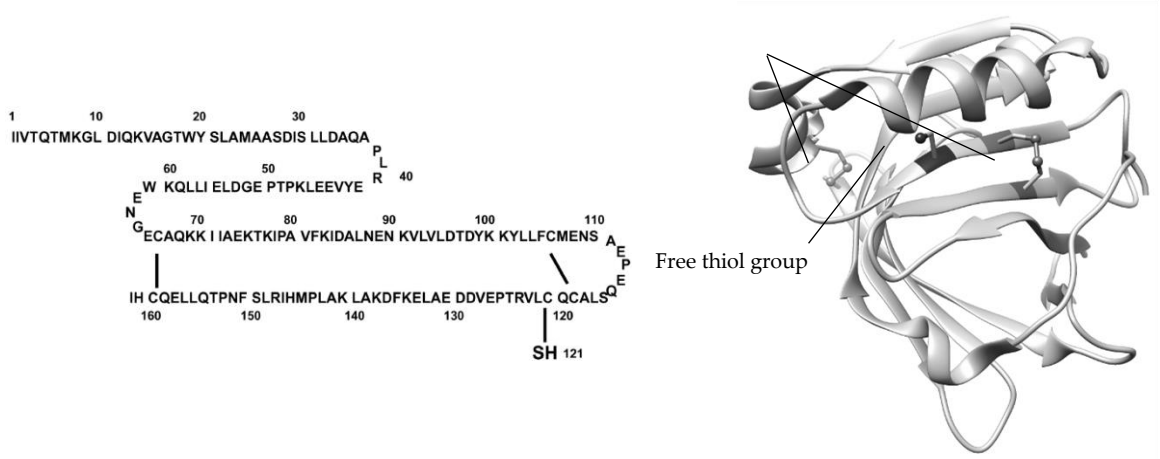
Figure 1.2 Sketch of the rennet coagulation of casein.

1.1.2. Whey proteins

The whey proteins make 17-19% of the total protein content in milk (Töpel, 2016). This section focuses on the main fractions β-lactoglobulin (56% of the whey protein fraction) and α-lactalbumin (21% of the whey protein fraction).

β-lactoglobulin has a molecular weight of 18,362 Da and is composed of 162 amino acids (Sawyer et al., 1999) (Figure 1.3 a). It is already known that the molecule consists of 6-15% α-helix, 38-52% β-sheet, 8-10% turn, and 32-35% random coil (de Wit, 2009; Tolkach and Kulozik, 2007). Hydrogen bonds, hydrophobic and electrostatic interactions stabilize the structure. Furthermore, β-lactoglobulin contains five cysteine residues with four forming intramolecular disulfide bridges (Cys^{66}-Cys^{160} and Cys^{106}-Cys^{119}) (Figure 1.3 b). The remaining free thiol group (Cys^{121}) is inside a hydrophobic pocket within the globular structure. In native state of β-lactoglobulin, Cys^{121} is not accessible.

Disulfide bridges



(a) (b)

Figure 1.3 (a) Primary structure of β-lactoglobulin with the disulfide bridges Cys^{106}-Cys^{119} and Cys^{66}-Cys^{160} and the free thiol group at position 121 (Liu et al., 2007); (b) 3D structure of β-lactoglobulin (Dombrowski, 2017 cit. after Papiz et al., 1986).

Depending on the pH, β-lactoglobulin forms monomers, dimers, or octamers. At the natural pH of milk in the range of 6.0-7.0, β-lactoglobulin exists as a stable, weakly negatively charged dimer. Between pH 4.0-5.0 (isoelectric point: pH 5.2) it is assumed to associate to octamers due to the reduced electrostatic repulsive forces between the molecules (Gottschalk et al., 2003). The monomeric β-lactoglobulin is present only below pH 3.0 and above 7.0 (Pessen et al., 1985). At pH values above 8.9, irreversible denaturation occurs (Roels et al., 1971).

The second major whey protein, α-lactalbumin, is composed of 123 amino acids and has a molecular weight of 14,174 Da. The secondary structure of α-lactalbumin contains 43% helices and 9% β-sheets (Pike et al., 1996), and four intramolecular disulfide bridges between Cys^{6}-Cys^{120}, Cys^{61}-Cys^{77}, Cys^{73}-Cys^{91}, and Cys^{28}-Cys^{111} (Permyakov and Berliner, 2000), which stabilize the tertiary structure. In contrast to β-lactoglobulin, α-lactalbumin does not have a free thiol group. Consequently, reactions causing structural rearrangements are often reversible (Kuwajima, 1996; Permyakov and Berliner, 2000; Schultz, 2000). α-lactalbumin has an ellipsoidal shape and can bind one calcium ion to each of its aspartic acid residues, thus connecting two domains of the molecule (Figure 1.4), which is strongly pH-dependent (Patocka and Jelen, 1991). At low pH, the calcium ion is solubilized and the structure of α-lactalbumin is thus weakened, and low temperatures are sufficient to denature this protein.

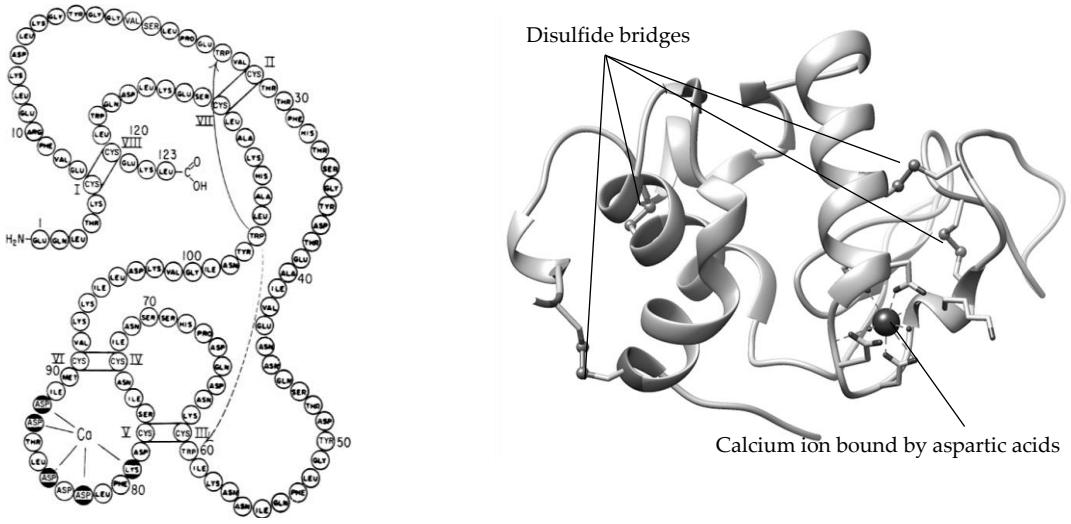


Figure 1.4 (a) Primary structure of α-lactalbumin with the disulfide bridges Cys^{6}-Cys^{120}, Cys^{61}-Cys^{77}, Cys^{73}-Cys^{91}, and Cys^{28}-Cys^{111}. The calcium-binding aspartic acids are marked in black (Farrell et al., 2002); (b) 3D structure of α-lactalbumin (Dombrowski, 2017 cit. after Brew, 2013).

The calcium-binding ability of α-lactalbumin increases its heat stability (Permyakov et al., 1985) and is thus, more heat denaturation resistant than β-lactoglobulin. The interactions between α-lactalbumin and calcium increase with increasing pH. In an acidic environment (pH < 4), the interactions are rather insignificant. At higher pH towards the isoelectric point of α-lactalbumin (4.8-4.5) (Farrell et al., 2004), the interactions increase resulting in the binding of one or two calcium ions (Patocka and Jelen, 1991).

External heat exposure causes the unfolding of β-lactoglobulin and the revealing of its hidden reactive thiol group. As a result, the β-lactoglobulin molecules build disulfide bridges among themselves, with κ-caseins on the micelle's surface, or with serum κ-casein, forming soluble whey protein aggregates or casein/whey protein complexes (Jang and Swaisgood, 1990; Smits and van Brouwershaven, 1980). The thermal denaturation enables a functionality improvement of β-lactoglobulin making it appropriate for various food applications (Tolkach and Kulozik, 2007), such as functional cheese powders. The mechanism of the thermal denaturation of β-lactoglobulin can be characterized as a multi-stage process (Figure 1.5). At temperatures above 40 °C, the native dimer (N_2) dissociates reversibly into native monomers (N). Between 40 and 55 °C, the monomers undergo an intramolecular transition into the `R-state´ (N_R), which is characteristic for pH values > 7.5.

The secondary structure of the molecule in the R-state is like in the native state and has only minor conformational differences of some side chains. However, the α-helix masking the free thiol group is involved as well, resulting in its better accessibility. Increasing the temperature above 60 °C, the protein partially unfolds (reversible reaction) and converts to the molton globule state (U_{MG}). From this point on, β-lactoglobulin is not able to refold anymore; this reaction is irreversible. Irreversible intermolecular reactions via hydrophobic bonds and thiol exchange lead to polymer and aggregate formation (U_n, U_m). A further temperature increase to 130-140 °C leads to a completely destructed secondary structure and hence, the irreversible denaturation of the β-lactoglobulin (U_D) (Tolkach and Kulozik, 2007).

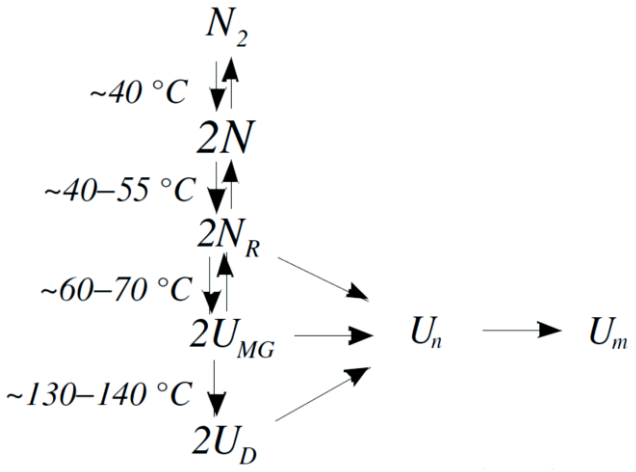


Figure 1.5 Mechanism of the thermal denaturation of β-lactoglobulin (Tolkach and Kulozik, 2007).

As mentioned before, β-lactoglobulin also reacts with κ-casein. The level of whey protein or casein association in skim milk depends on the heating conditions: A slow temperature increase results in 80% casein/whey protein complex formation (Corredig and Dalgleish, 1996; Smits and van Brouwershaven, 1980), a fast temperature increase made only 50% of the whey proteins complex with the casein micelles. The rest of the denatured whey proteins remained in the serum phase as disulfide-bonded/and or hydrophobically-associated aggregates (Oldfield et al., 1998; Singh and Creamer, 1991). The extent of casein/whey protein complex formation is also pH-dependent: In reconstituted skim milk at pH 6.5 for example, about 75-80% of the denatured whey proteins are associated with the casein micelle, whereas at pH 6.7, the association level is only 30% (Anema and Li, 2003). Figure 1.6 illustrates the formation of casein micelle/whey protein complexes and two proposed pathways for the formation of soluble casein/whey protein aggregates, since it is not clarified until now whether the dissociation of κ-casein from the micelles occurs prior (pathway II C, Figure 1.6) or after interacting with β-lactoglobulin (pathway II D, Figure 1.6).

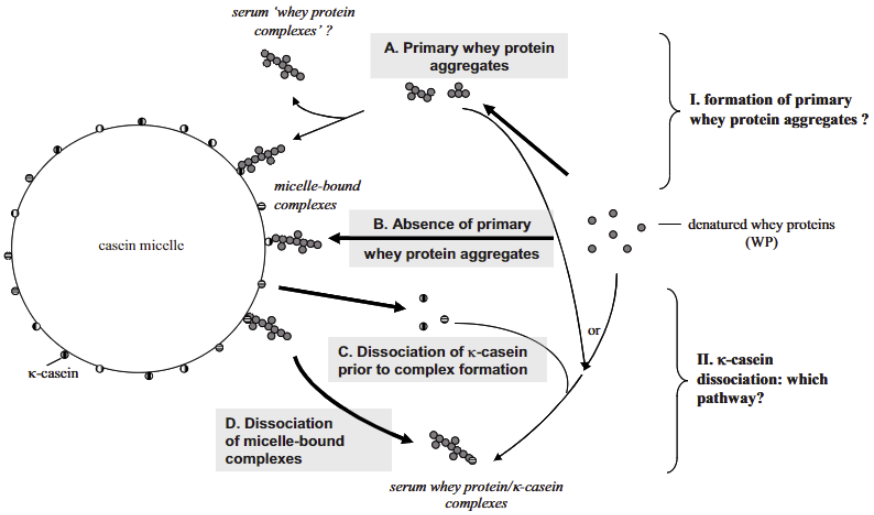


Figure 1.6 Schematic illustration of two currently proposed pathways for casein/whey protein complex formation in heated skim milk. I. Possible formation of primary serum whey protein; II. Dissociation of κ-casein as determinant pathway for the formation and properties of the serum casein/whey protein complexes (Donato and Guyomarc'h, 2009).

Furthermore, the size and internal structure of the aggregates also depends on the prevailing lactose content in the milk serum. In the presence of sugars, whey proteins prefer the associated form to avoid unfavorable water-protein interactions (Arakawa and Timasheff, 1982; Timasheff, 2002). Therefore, lactose protects β-lactoglobulin against denaturation if the lactose/protein ratio is high enough (Bernal and Jelen, 1985; Garrett et al., 1988; Jou and Harper, 1996; Plock et al., 1998; Spiegel, 1999).

The degree of whey protein denaturation depends on the time/temperature combination of the heat treatment and can be illustrated as so-called iso-effect lines (Figure 1.7). In general, the data depicted in this diagram allow to choose processing conditions during heating such that certain desired effects are achieved, while undesired effects are minimized or avoided. Regarding the degree of whey protein denaturation, heating milk at 100 °C for 100 s results in 60% whey protein denaturation, whereas at 100 °C for 1000 s the denaturation degree is > 90%. For functional cheese powders dealt with in this work, a high degree of denaturation was desired. Therefore, the time/temperature combination for the heat treatment had to be chosen in a region where only the whey proteins denatured in a high number without defecting any other milk constituents.

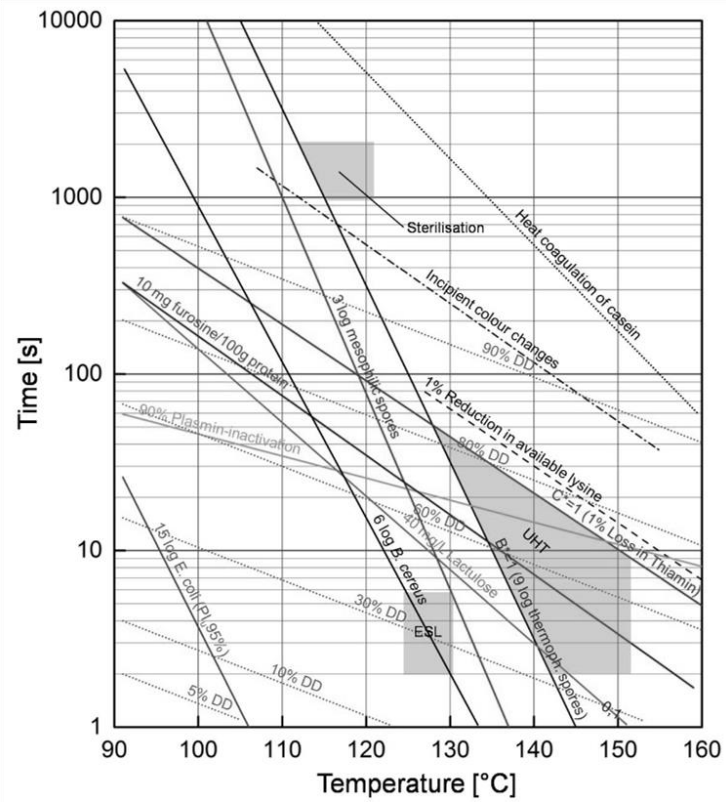


Figure 1.7 Iso-effect lines for the degree of whey protein denaturation, selected microbial inactivation, and occurring milk defects (Dumpler, 2018).

1.2. Milk protein concentration and fractionation and its implication on the rheological behavior of milk protein concentrates

The membrane separation process can be used for concentration or fractionation of milk components. Different membranes with different pore sizes and applied transmembrane pressures are commonly used in dairy industry. A membrane acts as a selective barrier through which targeted substances are retained in the retentate or transferred into the permeate. Consequently, fractions can be concentrated or depleted. In this work microfiltration (MF) and ultrafiltration (UF) are the relevant filtration techniques for functional MC and

MPC powder production. Figure 1.8 schematically illustrates the retention and permeation of casein micelles and whey proteins during MF and UF.

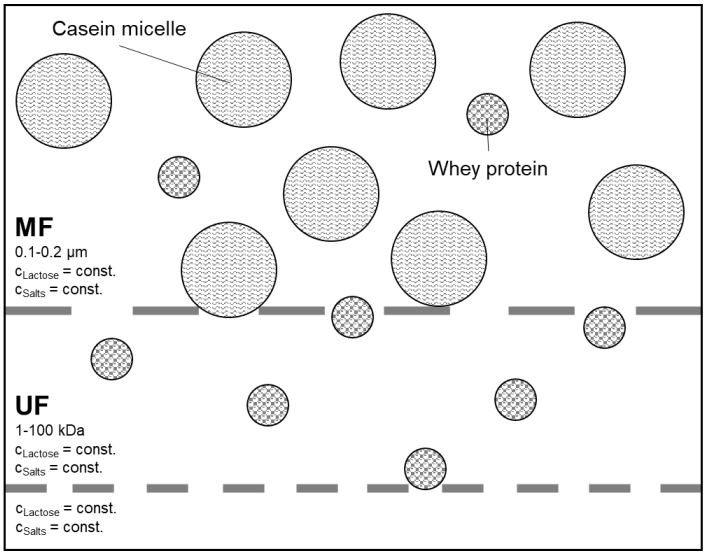


Figure 1.8 Schematic illustration of the retention and permeation of casein micelles and whey proteins during microfiltration (MF) and ultrafiltration (UF). Illustrations of the proteins are schematic and are not intended to meet the scale.

For MC production, MF is used for concentration. Due to the pore size of 0.1-0.2 µm, targeted casein micelles remain in the retentate, whereas whey proteins, lactose, salts, and water pass the membrane unhindered. It must be mentioned that with increasing MF filtration time the retained casein micelles accumulate on the membrane surface forming a deposit layer with a second retention effect, which reduces the flux and the whey protein permeation (Schopf et al., 2021). UF with a pore size of 1-100 kDa increases the total protein concentration, meaning the casein and whey protein fraction, and is hence, suitable for MPC production. The proteins remain in the retentate, whereas lactose, salts, and water pass the membrane.

During concentration, the concentration of the water-soluble constituents such as lactose and minerals remain constant in the soluble phase on retentate and permeate side. Hence, a certain amount of lactose and minerals remain in the retentate increasing the total solid content. In case of MF, whey proteins also remain partially in the retentate. Both soluble constituents and whey proteins reduce the powders' purity. An appropriate tool for washing out undesired milk constituents and for increasing the portion of the targeted components is filtration in diafiltration mode (DF). Using MF in diafiltration mode, up to 95% of the whey proteins can be washed out from the retentate (Nelson and Barbano, 2005).

The principle of diafiltration is keeping the retentate's volume constant during MF or UF by continuously adding diafiltration medium, such as UF permeate or water. The DF medium's choice depends on the desired final composition of the retentate. Whey protein-depleted MF retentates can be obtained by using UF permeate as DF medium, whereby the milk milieu remains unaltered. In case of lactose and mineral depletion, water is commonly used as DF medium. Figure 1.9 shows schematically an example of DF using MF.

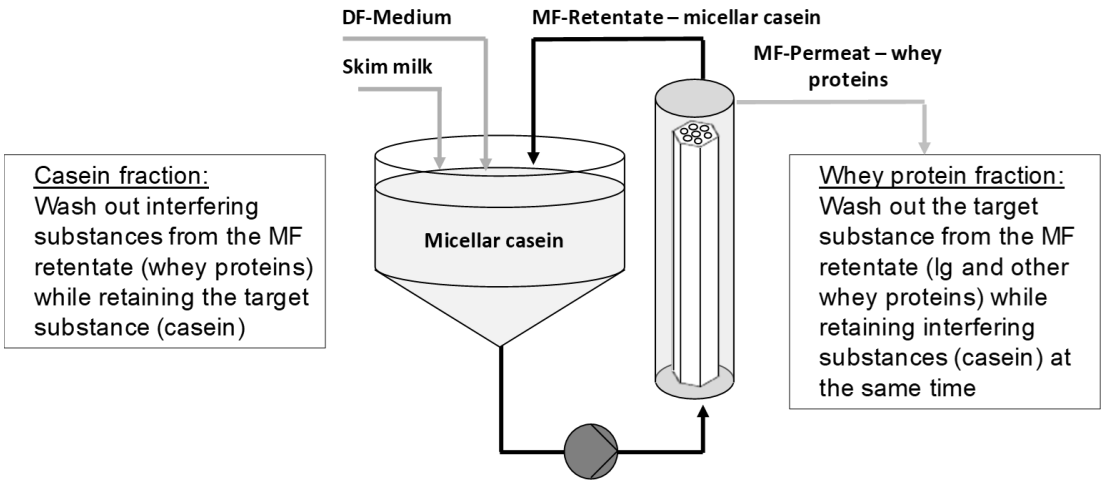


Figure 1.9 Schematic illustration of microfiltration in diafiltration mode (Heidebrecht, 2019).

MF in DF mode can be also used to adjust an exact casein/whey protein ratio, depending on the powder's application purpose: Skim milk powder (SM) contains all milk constituents in the same ratio as in milk and is commonly applied for yogurt production, as coffee whiteners (Maidannyk et al., 2020) or simply to increase the total solid content of milk for better product characteristics. MPC or MC contain different ratios of milk proteins in combination with different contents of lactose and minerals and are routinely applied, for instance, to increase the protein content of cheese milk to enhance the yield (Anema et al., 2006) or in the formulation of a wide range of food products such as high protein nutrition bars, meal replacements, ice cream (Agarwal et al., 2015; Parsons et al., 1985), bread (Gallagher et al., 2003), processed meat (Kilic, 2003), beverages, or medicinal nutrition products (Agarwal et al., 2015).

Filtration processes for constituents' removal or concentration affects the rheological properties of the concentrate. The viscosity plays an important role during milk processing e.g., in pumping or stirring operations; it is taken into account for the equipment selection for new food applications, unit operations and processing steps. Concerning the final product characteristics, the rheological profile of the intermediate offers the possibility to draw conclusions about the mouthfeel of the final product (Hermansson, 1975).The main factors influencing the viscosity are temperature, total solids, pre-heat treatment (temperature, holding time, and the resulting whey protein denaturation) and protein content (Bloore and Boag, 1981).

An additional, not negligible factor is the casein/whey protein ratio. On the one hand, the apparent viscosity depends on the volume fraction of the casein micelles, native, and denatured serum proteins; on the other hand, the apparent viscosity of the dispersion is high if the inherent viscosity of the continuous phase (lactose-dependent) is high as well (Anema et al., 2004; Jeurnink and de Kruif, 1993; Snoeren et al., 1982). The protein volume fraction can be raised by increasing the total protein concentration, which in turn, alters the protein voluminosity, or in other words, the protein hydration ratio (Nöbel et al., 2012). It is well-known that milk protein concentrates show shear-thinning behavior, which becomes more pronounced with increasing total solid contents. This means that at high total solid levels, the concentrate's structure rearranges to a less viscous state under shearing (Anema et al., 2014). The Power-law model (1.1) describes the viscosity of non-Newtonian fluids:

$$\eta = K \cdot \dot{\gamma}^{n-1} \tag{1.1}$$

If the flow behavior index *n* [-] is 0 < n < 1, the fluid is shear-thinning. For a Newtonian fluid is n = 1. We have shear-thickening behavior if n > 1.

During concentration via UF water containing lactose and salt is removed. The distance between casein micelles decreases; therefore, they interact more intensely, resulting in a higher apparent viscosity and a more shear-thinning behavior (Anema et al., 2014; de Kruif, 1997; Karlsson et al., 2005; Korolczuk, 1981). The same applies for micellar casein concentrates produced by MF: Sauer et al. (2012) and Solanki and Rizvi (2001) showed that the shear-thinning behavior of micellar casein concentrates increased with increasing concentration.

The rheological properties of non-Newtonian fluids can be expressed with the consistency coefficient *K* [Pa s^n] and the flow behavior index *n* [-]. Figure 1.10 shows exemplarily the development of *K* and *n* of reconstituted skim milk concentrates as a function of the total solid content.

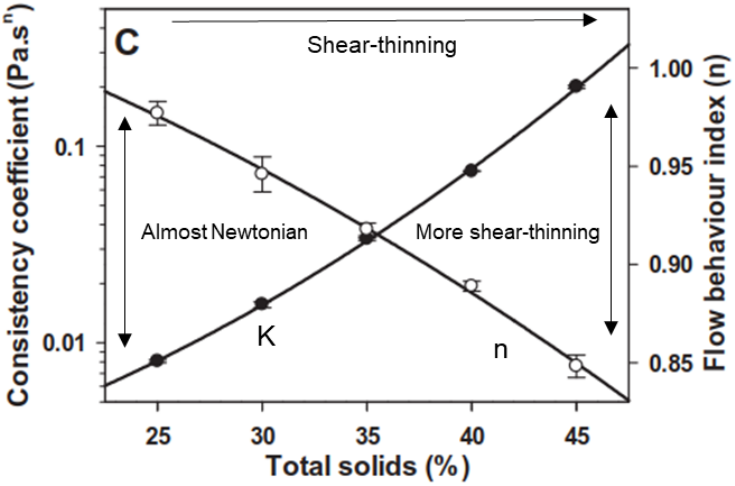


Figure 1.10 Consistency coefficient K and flow behavior index n of reconstituted skim milk concentrates at pH 6.7 (Anema et al., 2014, modified).

Numerous studies focus on only one main effect on the viscosity of milk protein concentrates, e.g., the total solid content (Anema et al., 2014; Bienvenue et al., 2003a; Solanki and Rizvi, 2001), the casein/whey protein ratio (Renhe and Corredig, 2018; Sauer et al., 2012), or the whey protein denaturation (Anema et al., 2014; Bienvenue et al., 2003a; Kessler and Beyer, 1991). However, these studies do not clarify whether and how the factors total protein concentration, casein/whey protein ratio and whey protein denaturation, which are variables studied in this thesis, interact with each other and whether the main effects or the factor interactions mainly affect the apparent viscosity.

The viscosity increase observed for unheated and heated milk protein concentrates with increasing casein/whey protein ratios can be explained by two effects: First, the interaction of casein micelles due to electrostatic forces and steric effects (de Kruif and May, 1991). In unheated concentrates, the negative charge of κ-caseins located on the micelle surface cause repulsive forces between the micelles (Dalgleish, 2007). In heated concentrates, denatured whey proteins, which are associated with the κ-caseins, interact predominantly (Dalgleish and Corredig, 2012). The second effect is the geometrical arrangement of the proteins and protein complexes differing in size. Polydispersity increases the volume fraction because small particles fill the gaps be-

tween bigger ones (Schaertl and Sillescu, 1994). Hence, casein/whey protein complexes and whey protein aggregates, which form during heating, induce a higher viscosity. These factors must be considered when producing functional MC and MPC powders for cheese production which is part of this thesis.

1.3. Principles of spray drying

During spray drying, a liquid feed solution containing dissolved and/or dispersed solids is sprayed into hot air at 180-220 °C. The surface area is very high due to the small droplet sizes atomized by a nozzle or a rotation disc. Evaporation of the water happens very quickly. The result are dry powder particles (Kim et al., 2009; Písecký, 2012). Figure 1.11 shows a schematic illustration of a spray dryer.

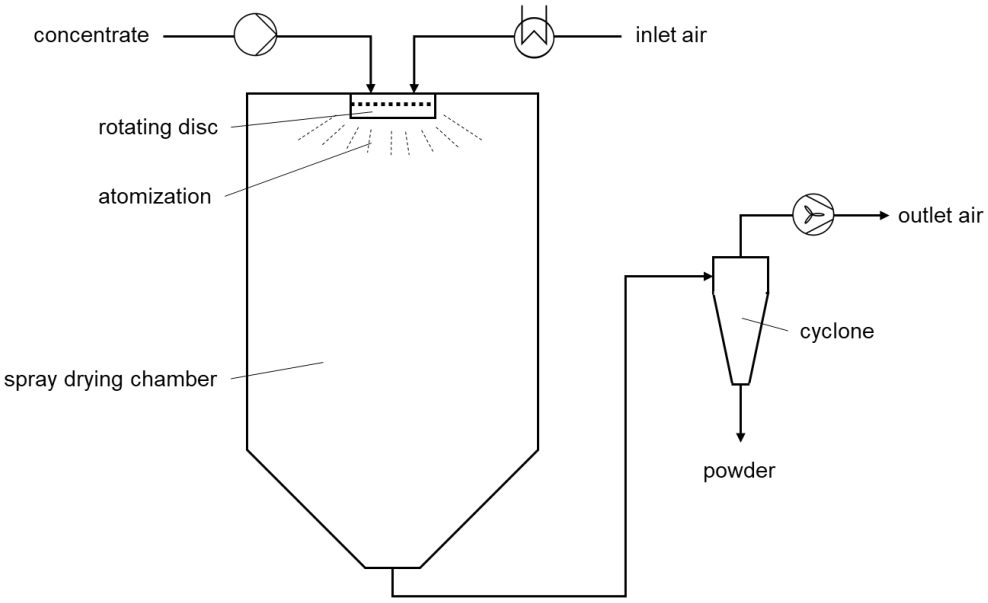


Figure 1.11 Schematic illustration of a spray dryer.

To better understand how the drying conditions affect a fluid droplet in a spray dryer, the changing drying air conditions are demonstrated by means of the Mollier h/x diagram (Figure 1.12).

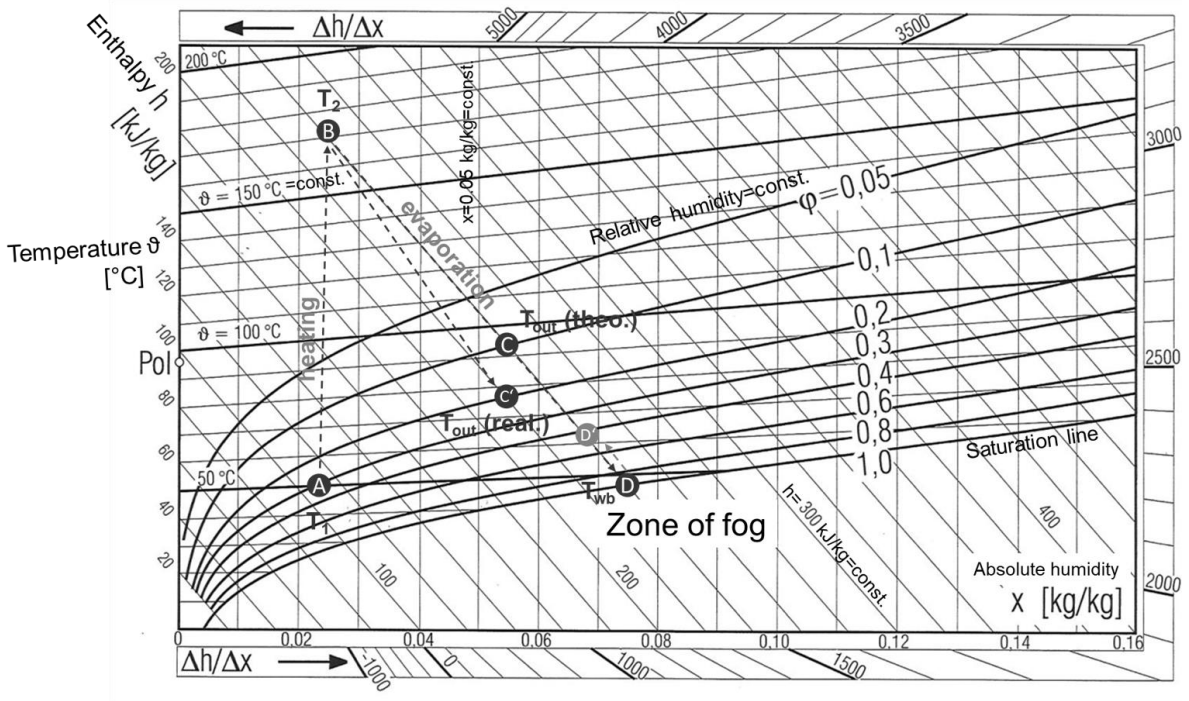


Figure 1.12 Mollier h/x diagram for humid air (Kessler, 1996; Gianfrancesco, 2009, modified).

The sequence of events can be described as follows:

- Heating of humid air: Inlet air with T_1, x_1, h_1, and φ_1 is heated up to T_2 (A→B). T_2 is the dry bulb or the air inlet temperature (temperature of not water-saturated air). After T_2 is reached, the feed is pumped into the spray dryer and atomized.
- Drying: During this phase, the temperature decreases while the relative humidity and absolute humidity increase (B→C). Theoretically drying takes place under adiabatic conditions but due to some heat losses, the drying is not isenthalpic—the outlet air temperature T_{out} is lower and φ_{out} is higher in reality (B→C').
- The surface water activity a_w of the solution drops is equal to 1 (free water) at the beginning of drying. This implies that the air layer around the droplet, which is directly in contact with its surface, has a relative humidity of 100% ($\varphi = 1$) and the so-called wet bulb temperature T_{wb} (D).
- First, the drop reaches almost immediately T_{wb}; second, drying takes place constantly; third, if the drying particle reaches a critical moisture content, the surface is no longer saturated and the a_w decreases. As a result, if the drying air is hotter than necessary for evaporation, the drop surface temperature could increase (D→D') (Gianfrancesco, 2009).

The drying of a droplet takes place in two steps as shown in Figure 1.13. At the beginning of the first period, the so-called constant rate, the droplet is still a fluid having the moisture content of the feed and an a_w-value of 1. At this stage, free water evaporates from the droplet's surface. Due to its fluid character, moisture can migrate easily from the droplet's interior to its surface. As long as the conditions are saturated, water constantly evaporates without exceeding the wet bulb temperature until evaporation is completed whereby

the particle shrinks. After the constant drying rate period, the particles reach a critical point where suddenly a moisture gradient across the droplet diameter occurs. Here, the droplet turns from a fluid to a wet solid. The critical moisture content for milk products lies between 30 and 15%. The falling rate or second drying rate period follows. From that point on, the moisture diffusion rate through the particle controls the drying. More heat than mass is transferred and the particle heats up faster (see also Figure 1.12, D→D'). Moisture and temperature gradients are in the interior of the particle and the surface forms a crust (Birchal et al., 2006; Charlesworth and Marshall, 1960; Kim et al., 2009; Písecký, 2012).

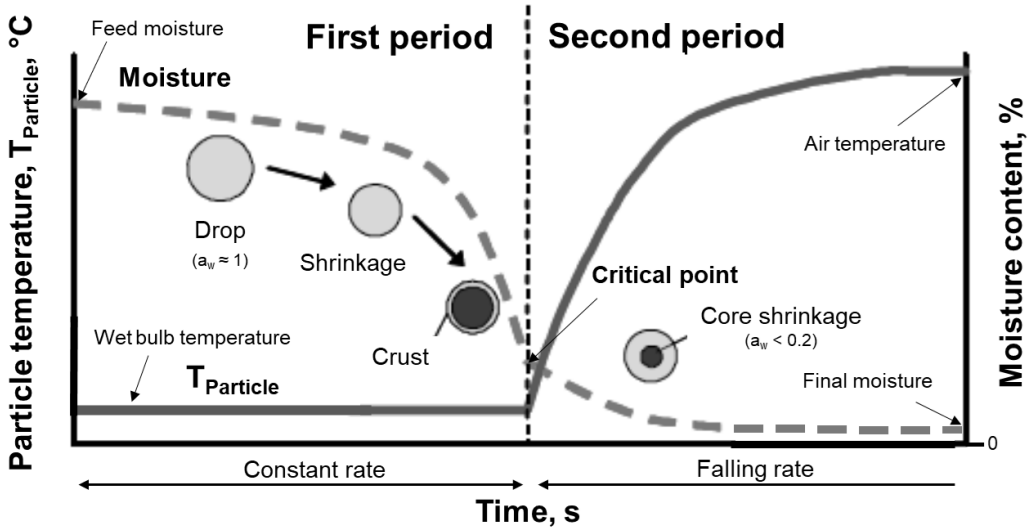


Figure 1.13 Schematic illustration of the droplet temperature and moisture content evaluation during spray drying (Kim et al., 2009, modified).

According to Písecký (2012), the particle temperature is equal to the outlet air temperature when equilibrium is achieved between the particle and the drying air ($a_w = \varphi$, $T_2 = T_p$). It should be noticed that droplets of milk concentrate have a lower water activity than 1 (~0.85-0.90). Therefore, the temperature during drying will be higher than T_{wb}. For the milk droplet temperature, the author found a relationship according to Equation (1.2).

$$T_p = T_2 - (T_2 - T_{wb}) \cdot \frac{(a_w - \varphi)}{(1 - \varphi)} \tag{1.2}$$

In two or three stage drying, an integrated/external fluid bed or belt is placed at the outlet to further reduce the moisture content of the powder. In single stage drying, only the heated air during spray drying removes the water. Hence, the drying air has to be hot enough to achieve the desired moisture content of the final product. It should be considered whether the last stage of drying, where the outlet temperature is high and the moisture content low, impairs the product quality. Keeping the concentration of the feed solution and the inlet temperature low can prevent from overheating. Even though a two stage drying is more economic, it is not applicable for certain thermoplastic or hygroscopic products, which get too sticky at higher moisture contents (Písecký, 2012).

1.4. Solubility of milk protein concentrate powders

Stored high protein powders like MPC or MC usually show a deterioration in the rehydration properties and protein solubility (Carr and Golding, 2016). This possibly requires modification of existing unit operations or product formulations for a better powder rehydration (Crowley et al., 2015).

The caseins' structure and hence, their rehydration properties, is influenced by the composition of the aqueous bulk solution and the processing conditions during powder manufacture (Felix da Silva et al., 2017). In addition, the powder storage conditions, the particle structure, and the rehydration conditions have an impact on the powder solubility too (Barbosa-Cánovas et al., 2005; Gaiani et al., 2009, 2006a). Crowley et al. (2015) explained the poor solubility with the mineral depletion during DF, since powders with high protein contents produced in DF mode, show a higher Ca^{2+}-activity due to the changes in milk salt equilibrium between the dispersed and the aqueous phase, which in turn, renders the powder less soluble (Baldwin, 2010; Crowley et al., 2014). Not only the milk salts, but also lactose plays an important role during drying. Carbohydrates replace the removed water molecules via hydrogen bonds and keep thus, the native structure of dried proteins (Allison et al., 1999, 1996; Prestrelski et al., 1993). As sterical spacer and by hydrogen bonding to the amino acid chain, lactose prevents protein interactions (Baldwin, 2010).

The caseins are considered as the main components poorly dissolving in MPC85 (Anema et al., 2006). Mimouni et al. (2010) observed increased interactions between the casein micelles during powder storage resulting in compaction of micelles and the formation of a closely packed micelle surface layer. This closely packed monolayer micellar `skin´ was also observed by McKenna (2000). This author made cross-linking of micelles by non-micellar proteins and the close association of micelles at the surface responsible for the observed effect. According to Havea (2006) these are hydrophobic protein-protein interactions, which enhance the formation of poorly dispersible aggregates. The best wetting properties show powders with a high lactose and/or a low lipid coverage on the particle surfaces (Fäldt and Bergenståhl, 1996; Gaiani et al., 2006a; Kim et al., 2002).

Mimouni et al. (2009) evidenced with light and scanning electron microscopy that commercial MPC85 powder particles contain large vacuoles. They found that the volume of undissolved powder particles gradually decreased over rehydration time, but single casein micelles were not released. Based on their findings, the authors proposed a mechanism for MPC85 rehydration. They hypothesized that the vacuoles fill with water first before the proteins in the surrounding outer shell dissolve due to erosion and collapse at a later stage (Figure 1.14). Therefore, it is advisable to take a certain amount of time for MPC85 rehydration into account.

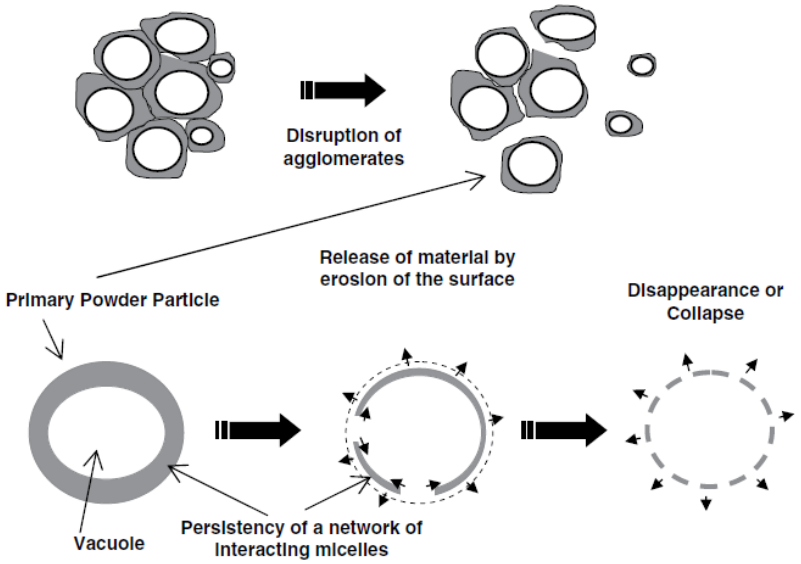


Figure 1.14 Hypothesized rehydration process of MPC85 (Mimouni et al., 2009).

Light microscopy images by Crowley et al. (2015) showed that with increasing protein content in MPC powders, the air vacuoles and the protein-rich skins on the particles' surfaces (dark regions surrounded by distinct white regions) became more prominent (Figure 1.15). This should affect the resolubilization of the powders with different protein compositions, which is part of the investigations covered by this thesis.

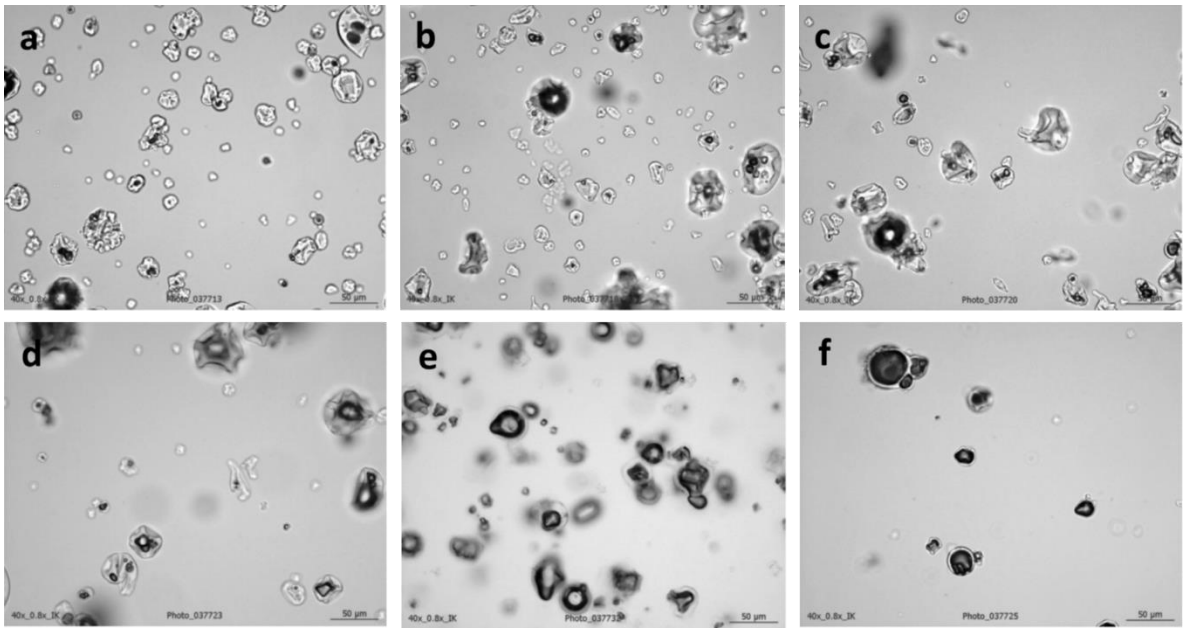


Figure 1.15 Light microscopy images of MPC35 (a), MPC50 (b), MPC60 (c), MPC70 (d), MPC80 (e), and MPC85 (f) (Crowley et al., 2015).

Furthermore, the powder rehydration is temperature- and shear-dependent. Higher temperatures lead to shorter rehydration times due to an increased water transfer towards the inner core of the powder particles (Anema et al., 2006; Felix da Silva et al., 2017). Low temperatures are known to improve water absorption into the casein micelle and the release of calcium phosphate and β-casein, which leads to a partial loss in micellar

integrity and, thus, to a more porous structure (Gaucheron, 2005), which may improve the solubility compared to ambient temperatures.

Various shear treatments are used for powder redispersion. In most of the studies a simple magnetic stirrer system was used with different filling heights and stirrer bar dimensions (Crowley et al., 2015; Kieferle et al., 2019; Lin et al., 2018; Martin et al., 2010) or overhead stirrers equipped with different stirrer blade geometries (Anema et al., 2006; Crowley et al., 2015; Havea, 2006; Mimouni et al., 2010; Schokker et al., 2011). For standard milk powders, e.g., skim milk, this has been shown to be sufficient, but for poorly soluble powders such as MPC or MC, these methods are most likely not appropriate. The judgement of good or inferior rehydration behavior may therefore well depend on the mixing system used in the respective studies. Chandrapala et al. (2014) examined the effect of low shear (overhead stirrer), high shear (handheld homogenizer), ultrasonication, and high pressure homogenization (HPH) on the solubility of MPC80 and MC. The authors showed that even high shear rotor/stator systems (Ultraturrax) compared to low shear does not significantly downsize the powder particles of MPC80 and MC at the same input energy density. Only HPH (120 and 200 bar) shifted the particle size distribution towards casein micelle size. However, the applied pressures were also insufficient to achieve a monomodal particle size distribution around ~0.2 µm. The lowest necessary shear impact for full MPC80 and MC dissolution has not been identified. Moreover, an Ultraturrax does not run continuously, making it hard to transfer to industrial shear systems. In the dairy industry, two high shear units are widely implemented and can be used for powder redispersion: shear pumps like in powder mixers and HPH. The shear conditions, such as shear rate, turbulent flow, and cavitation (only in HPH) are involved in particle destruction. Such shear conditions may be appropriate to fully solubilize poorly soluble high protein powders like MPC and MC.

1.5. Spray drying of lactic acid bacteria in milk matrices

Spray drying lactic acid bacteria is a common way to encapsulate bacteria in certain matrices. For this, a suitable stabilization technology, an appropriate drying method and product matrix should be considered (Poddar et al., 2012). Khem et al. (2015) showed that that milk matrices are suitable for integrating bacteria in a protective environment. They reported that whey protein isolates (WPI) and skim milk have a higher protective effect on *Lactobacillus plantarum* during drying than carbohydrates such as lactose or trehalose. The crust formation in the first drying period creates a shell and thus, prevents the droplet from overheating in the second drying period. Moreover, the calcium present in milk was postulated to increase the intrinsic heat resistance of the lactic acid bacteria. Desmond et al. (2002) could show that adding 0.3 M sodium chloride to a suspension of reconstituted skim milk (20% (w/v)) and *Lactobacillus paracasei* resulted in a high degree of cross-protection for the bacteria against heat stress during spray drying.

Würth et al. (2016) developed a spray drying process to encapsulate probiotics in milk matrices with the classic rennet gelation process for cheese manufacture (Figure 1.16). Skim milk containing *Lactobacillus paracasei* ssp. *paracasei* F19 was cold-renneted at 4 °C prior spray drying, where aggregation of the para-casein micelles does not occur (Bansal et al., 2008, 2007). Such so-called `capsule precursor powder´ formed the final water-insoluble hydrogel capsules upon rehydration at temperatures above 16 °C.

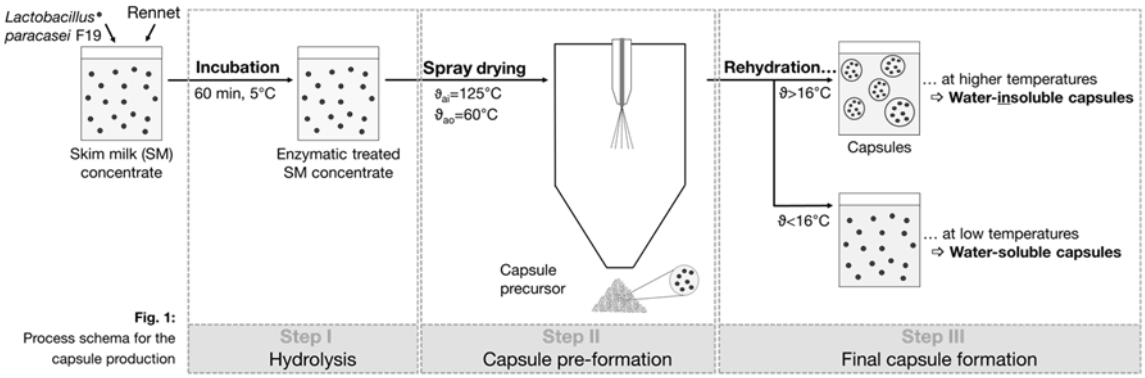


Figure 1.16 Process scheme of milk protein microcapsules production. Step I: Hydolysis; step II: Capsule pre-formation during spray drying; step III: Final capsule formation upon rehydration (Würth et al., 2016).

Khem et al. (2016) investigated the effect of denatured whey proteins on the survival of *Lactobacillus plantarum* spray-dried in WPI, lactose, or WPI/lactose mixtures. The survival rates in WPI were significantly higher than in lactose or WPI/lactose mixtures. This observation led the authors to assume that the whey proteins have a protective effect on the bacteria. They assumed that the whey proteins unfold during spray drying at an outlet temperature of approximately 70 °C and that these interact hydrophobically with the hydrophobic bacteria resulting in aggregate formation. Consequently, the microorganisms embed in the capsule and are protected against inactivation during spray drying. Based on these results it would be obvious that heated WPI solutions may have a better protective effect on bacterial cells due to the higher whey protein denaturation degrees. This was done by Picot and Lacroix (2004), who encapsulated *Bifidobacterium breve* in heated WPI solutions (80 °C/30 min). They achieved survival rates of 26% (corresponding to 10^9 cfu mL^{-1}, cfu: colony forming units) when spray drying at an air inlet and outlet temperature of 160 °C and 80 °C, respectively. This knowledge can be used to develop novel powder-based cheese products, containing alive lactic acid bacteria, which rapidly activate upon rehydration.

1.6. Incorporation of whey proteins in cheese by heat treatment

Incorporating whey proteins into cheese has advantages, such as the higher nutritional value, the increased cheese yield and the sensory improvement of low fat cheese (Hinrichs, 2001). Whey proteins can be included into the cheese matrix in native or denatured form. For the latter, there are four possibilities:

1. High heat treatment to bind the whey proteins to the casein micelle's surface;
2. Combination of high heat and membrane techniques to concentrate the aggregated and denatured whey proteins as well;
3. Adding microparticulated proteins to the cheese milk or
4. Adding microparticulated proteins to the cheese matrix (Hinrichs, 2001).

The cheese yield is defined according to Equation (1.3).

$$Yield = \frac{m_{cheese}}{m_{milk}} \cdot 100 \qquad (1.3)$$

The yield can be increased by increasing fat and protein contents, incorporating whey proteins; or by adding lactose, ash, or water. In fresh cheese production, it is common to include the whey proteins to achieve a higher cheese yield. There are three different processing options for incorporating whey proteins into fresh cheese by heating (Figure 1.17).

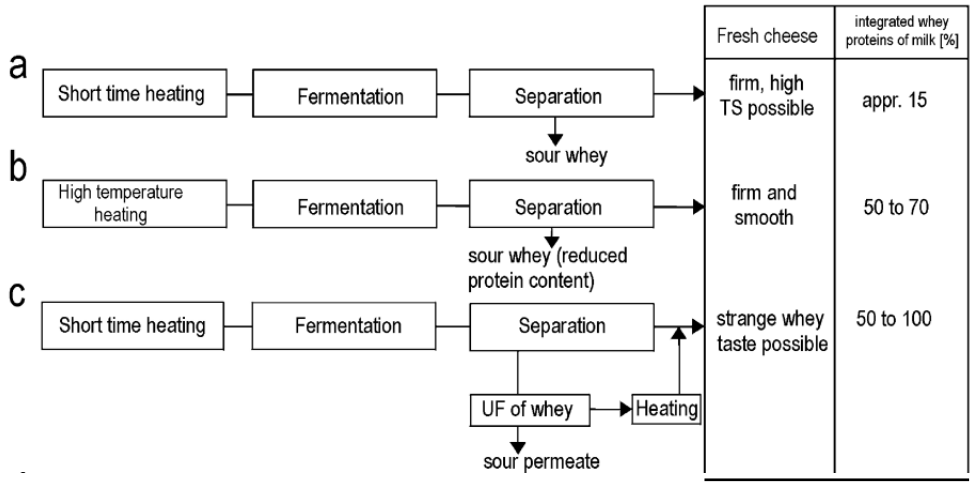


Figure 1.17 Three ways to incorporate whey proteins by heat treatment in fresh cheese production (Hinrichs, 2001, modified).

Traditionally, skim milk is heated to 74 °C for 40 s before fermentation. Subsequently, a centrifugal separator removes the whey. The resulting cheese is firm due to high total solid contents including only ~15% of the whey proteins (Figure 1.17 a). Process b, which is the so-called `thermoquarg´ process, replaces the short time heating with high temperature heating (82-95 °C for 360-80 s) to denature the whey proteins and bind them on the casein micelles' surfaces (Figure 1.17 b). The advantages are the higher cheese yield and the better water binding, which results in a firm and smooth texture. This process allows to include 50-70% of the WP into the cheese matrix. Another way to increase the cheese yield is the recycling of acid whey in concentrated and heated form into the cheese curd (Figure 1.17 c). At too high concentrations, a bitter taste can occur during storage (Hinrichs, 2001).

Whey protein particles are not an integral part of the structure forming cheese matrix, but they are sterically retained in the pores of the coagulated casein micelle structure. The structure of cheese containing microparticulates or whose milk was high heat treated, is schematically illustrated in Figure 1.18.

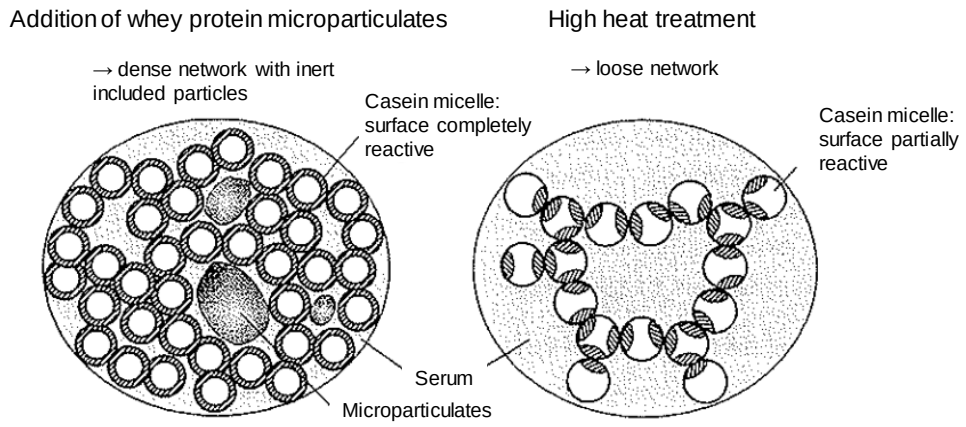


Figure 1.18 Schematic representation of rennet gel structures from milk containing microparticulated whey proteins and from high heated milk (Steffl, 1999, modified).

However, this work focuses on the whey protein incorporation by heat treatment only. There are some studies dealing with high heat treatment of cheese milk. Steffl (1999) reported on an impaired rennet gelation,

if more than 60% of the β-lactoglobulin was denatured and bound on the casein micelle surfaces. Then, the interaction of the rennet enzyme with the κ-casein was disturbed due to the partly occupied binding sites. In contrast to Steffl (1999), Anema et al. (2007) found that the observed retarded rennet gel formation was irrespective of whether denatured whey proteins were associated with the casein micelles or self-aggregated in the serum phase. Thus, these authors assumed that these complexes inhibit further aggregation and therefore, retard the gelation.

However, these detrimental effects of present whey protein aggregates on rennet gelation can be circumvented by UF concentrating or increasing the casein/whey protein ratio, i.e., by reducing the whey protein content by MF prior to heat treatment. Schreiber (2000) and Schreiber and Hinrichs (2000) reported that at casein concentrations above 8% the gel strength of heated UF concentrates was higher than the gel strength of pasteurized skim milk, even if all whey proteins were denatured. The integration of denatured whey proteins into the cheese matrix seems therefore possible if the casein concentration is high enough. Figure 1.19 shows the correlation between casein concentration, casein occupancy by denatured whey proteins, and the gel strength. At low casein concentrations, the gel strength decreases with increasing whey protein denaturation. Concentrating the casein fraction overcomes this effect.

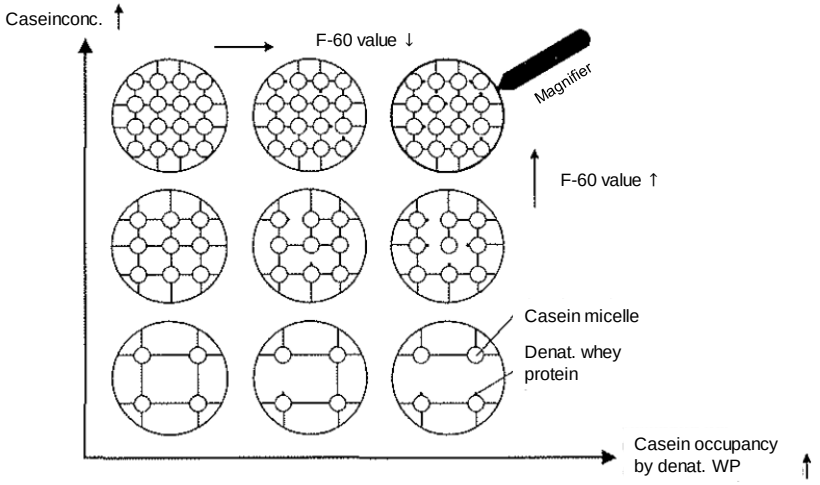


Figure 1.19 Correlation between casein concentration, casein occupancy and gel strength (F-60 value) (Schreiber, 2000, modified).

Bulca (2007) found that skim milk with casein/whey protein ratios of 3.4 : 0.01% or 6.4 : 0.65% ultra-high heat-treated (UHT) at 140 °C for 10 s gelled as fast as pasteurized skim milk and showed the same gel firmness (Figure 1.20). Both criteria were related to the standard cheese making process. In other words, a coagulation time/gel firmness of value 1 means that the same data were obtained for the standard process and the process using UHT-treated milk.

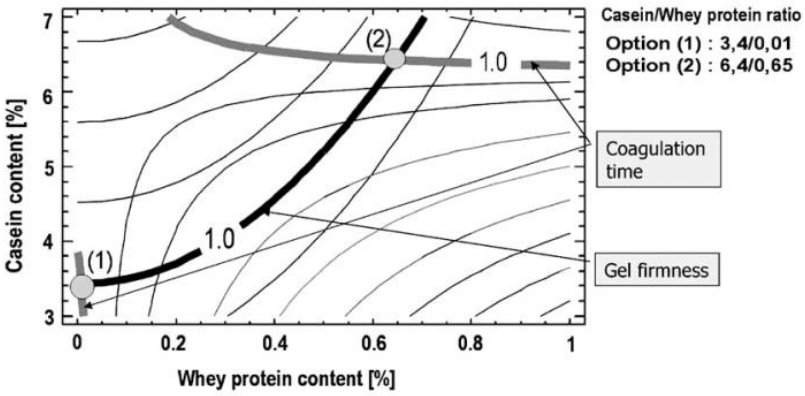


Figure 1.20 Iso-effect lines of coagulation time and gel strength of UHT cheese milk (Bulca et al., 2001).

Steffl (1999) could show in pilot plant studies that heating the cheese milk for soft cheese production can potentially increase the yield up to 25% (Figure 1.21).

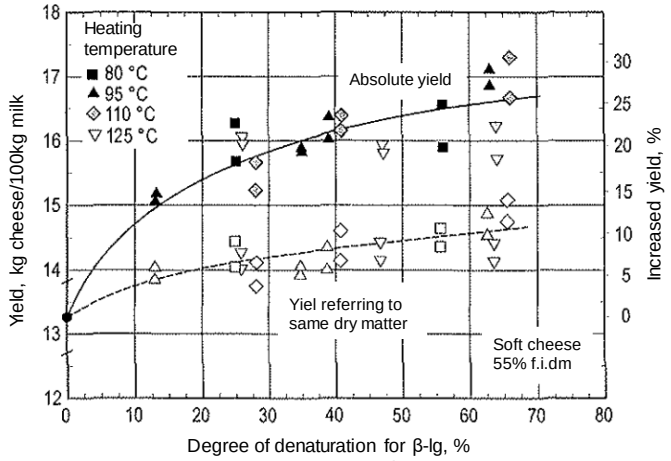


Figure 1.21 Soft cheese yield from high heated milk (Steffl, 1999, modified).

The desired cheese type and texture decide upon the whey protein incorporation process. Some processes—like UHT treatment, UF, or addition of microparticulated whey—are already established in dairies (Hinrichs, 2001). This allows to create innovative cheese products—such as powder-based wheyless cheese.

2. Objective and outline

As already described, milk protein powders have been subject to wide-ranging investigations within the last several decades. In this context, improvement of the general understanding of the impact of proteins' structural attributes in combination with processing conditions on the powders' solubility was and still is a primary objective of scientific and industrial research. Further to that, reconstituted concentrates are commonly used in scientific works as if they were substitutable to fresh milk concentrates. However, various low or high shear tools for redispersion were used in these studies and specific single dairy powders were determined at different temperatures, concentrations, and aqueous phase compositions. To the best of my knowledge, a study comparing the various prevalent milk powders in industrial use with their different compositions and powder properties has not been conducted so far.

The focus of this work was on high shear treatments such as rotor/stator mixing systems and high pressure homogenization and its implications on the rheological and functional properties in terms of rennet gelation. The powders were either dissolved in water to compare their rheological profile to fresh concentrates or in milk—as this is routinely applied in cheese manufacture to increase the protein content—to evaluate the impact of remaining powder particles on the rennet gelation behavior.

Based on that, a concept for powder-based cheese manufacture was developed. The idea was to produce powders of unheated and heated cold-renneted skim milk concentrates forming gels upon rehydration. The integration of the whey proteins in the cheese matrix avoids whey protein drainage should be resulting in a high yield, i.e., a higher amount of cheese from a certain amount of milk. Since heating has a detrimental effect on the renneting properties of the casein micelles as described before, the total protein concentration was increased and the casein/whey protein ratio was varied to overcome this effect.

However, concentration inevitably affects the concentrate's viscosity—particularly after heat treatment. Another research question was, therefore, which macroscopic mechanisms are responsible for changes in viscosity upon increasing the total protein concentration, varying the casein/whey protein ratio, and heating.

The aim of this thesis was on the one hand, to comprehensively study the solubility of milk protein powders of different composition dissolved in water and milk under variable processing conditions and its implications on the flow and rennet gelation behavior. The hypothesis was that rehydration conditions and composition of milk protein powders can lead to differences in solubility and presence of powder aggregates due to inaccessible proteins in the powder agglomerates and thus, to variation in the resulting viscosity and rennet gelation behavior, which could be disturbed by large powder particles.

On the other hand, an `instant cheese powder´ for wheyless cheese production was developed in the context of which the flow properties of fresh milk protein concentrates for the powder production were investigated. Considering that casein/whey protein complexes and whey protein aggregates form during heating, it was hypothesized that the viscosity of the feed solutions can be adapted by taking these effects into account. Further to that, the cold-renneted skim milk ultrafiltration (UF) and microfiltration (MF) powders should result in a homogeneous gel matrix upon rehydration at temperatures below 16 °C with a high yield and a high survival rate of integrated lactic acid bacteria due to the protective effect of the whey protein aggregates.

The experiments comprised the dissolution of milk protein powders in rotor/stator mixing systems and high pressure homogenizers on a laboratory and pilot scale as well as UF and MF in diafiltration mode, and spray drying on a pilot scale for instant cheese powder production.

Assessment criteria were particle size measurements, rheological measurements, serum phase/sweet whey analyses, such as the determination of its amount and composition, and determination of the viable cell count. The casein, whey protein, and lactose content as well as the degree of whey protein denaturation were determined by reversed-phase high performance liquid chromatography.

As a result of this work, insights into the effect of incomplete rehydration of dissolved milk protein powders of various composition on the flow properties and rennet gelation behavior should be gained. With upscaling experiments, we can recommend suitable shear conditions for best powder dissolution and protein functionality on a laboratory as well as on a pilot scale. Furthermore, a basis for instant cheese powder production containing a high number of viable cells and all required ingredients forming cheese, but without whey protein drainage is to be created.

3. Results

3.1. Impact of temperature and high pressure homogenization on the solubility and rheological behavior of reconstituted dairy powders of different composition

Summary and contribution of the doctoral candidate

Investigating the solubility and rheology of milk protein powders is of great interest because they are commonly used in research or industrial applications as substitute for fresh milk concentrates. Highly concentrated protein powders are particularly challenging in terms of their solubility.

Therefore, the aim of this study was to compare the solubility of milk protein powders with different compositions in a controlled study under the same experimental conditions in a range covering even poorly redispersable milk protein powders. Specifically, the impact of temperature at low shear (4, 20 and 50 °C) and high pressure homogenization (50 °C, 500 bar) on the resulting rheological properties of skim milk powder (SM), milk protein concentrate powder containing 50% (w/w) protein (MPC50), milk protein concentrate powder containing 85% (w/w) protein (MPC85), and micellar casein powder (MC) was assessed, which has not been studied in a direct comparison so far. As criteria, the rheological properties and particle sizes of reconstituted and fresh concentrates with same composition were compared.

It turned out that the temperature determines the rehydration time. Similar particle size distributions were obtained after stirring at 4 °C overnight and 50 °C after 45 min. Samples stirred at 20 °C overnight still contained powder particles of 10 μm and larger. This temperature is not a good choice as powder rehydration temperature if reproducible product characteristics are desired.

Powder composition impacts the powder aggregate's size. Increasing the casein content combined with a lactose reduction decreases the powder dissolving ability leading to bigger particles, higher shear stresses and stronger shear-thinning. Depending on the powder type, the particle sizes and shear stresses increased as follows: SM < MPC50 < MC < MPC85. SM and MPC50 were already fully rehydrated after 45 min at 50 °C. On the contrary, highly concentrated protein powders like MPC85 and MC required a high pressure homogenization step at 500 bar to disintegrate all powder aggregates to single particles, i.e., to casein micelle size ($d_{50,3}$ = 0.124 μm). This drastically decreased the shear stress of MPC85 and MC.

After full rehydration of the powders, no differences in terms of particle sizes and rheology between the reconstituted and fresh concentrates were observed. It was concluded that the particle size is the most important impact factor in the rheological properties. The results show that if the rehydration conditions are adapted to the powder type, fresh concentrates are completely substitutable with reconstituted concentrates regarding their rheology.

The most significant contribution to this manuscript was made by the doctoral candidate. This comprised the conception and design of experiments based on preceded critical literature review as well as major conduction of data analysis, data interpretation, and discussion. In addition, writing and editing of the manuscript was done by the doctoral candidate. The co-author contributed to the project outline, the discussion of results, and the revision of the manuscript.

Adapted original manuscript[1]

Impact of temperature and high pressure homogenization on the solubility and rheological behavior of reconstituted dairy powders of different composition[2]

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Abstract: Investigating the solubility and rheology of milk protein powders is of great interest because they are commonly used in research or industrial applications as substitute for fresh milk concentrates. The solubility of skim milk (SM), milk protein concentrate with 50% and 85% protein (MPC50, MPC85) and micellar casein powder (MC) was evaluated at variable temperatures (4, 20 and 50 °C) and their resulting rheological profile was compared to freshly produced samples. Powder composition impacts the powder aggregate's size. Increasing the casein content combined with a lactose reduction decreases the powder dissolving ability leading to bigger particles, higher shear stresses and stronger shear-thinning. Highly concentrated protein powders like MPC85 and MC required a high pressure homogenization step to disintegrate all powder aggregates to single particles, i.e., to casein micelle size. If the rehydration conditions were adapted to the powder type, fresh concentrates were completely substitutable with reconstituted concentrates regarding their rheology.

Keywords: Milk protein powders; Milk protein concentrates; Rehydration; Dissolution; Particle size; Viscosity

3.1.1. Introduction

Milk and whey are dried in huge amounts and reconstituted prior to the manufacture of various dairy and other food products. Due to differences in functionality and availability of suitable technologies such as micro- (MF) and ultrafiltration (UF), either alone or both combined in diafiltration (DF) mode, milk powders exist in various forms differing in protein composition and content of low molecular weight constituents. The presence of minerals or lactose mainly depends on the composition of the DF medium. If water is used, these components get washed out, upon use of milk UF permeate (milk serum) the aqueous phase composition remains unchanged. This in turn, is a decisive factor for the redispersability or solubility (used here interchangeably) of dairy powders. Skim milk powder (SM) contains all milk constituents in the same ratio as in milk. It can routinely be applied for yogurt production, as coffee whiteners (Maidannyk et al., 2020) or simply to increase the total solid content of milk for better product characteristics. Milk protein concentrate (MPC) or micellar casein powders (MC) contain different ratios of milk proteins in combination with different contents of lactose and minerals. These powders can be used, for instance, to increase the protein content of cheese milk to enhance yield (Anema et al., 2006) or in the formulation of a wide range of food products such as high

[1] Adaptions refer to formatting issues: e.g., numbering of sections, figures, tables, and equations; abbreviations, manufacturer specifications, axis labeling, figure captions, and style of citation. References are listed at the end of this thesis, combined with the references of the other publications, to avoid redundancies.

2 Originally published in: Powder Technology (2020), Vol. 367, pp. 285-295. Permission for reuse of this article was granted by Elsevier.

protein nutrition bars, meal replacements, ice cream (Agarwal et al., 2015; Parsons et al., 1985), bread (Gallagher et al., 2003), processed meat (Kilic, 2003), beverages or medicinal nutrition products (Agarwal et al., 2015).

The main problem with highly concentrated protein powders like MPC or MC is their low solubility and insufficient dissolving ability (Carr and Golding, 2016). This requires modification of existing unit operations or product formulations for a potentially better powder rehydration (Crowley et al., 2015). A large number of studies has reported on the solubility of dairy powders. For instance, Anema et al. (2006) and Mimouni et al. (2009) identified the dissolution of casein as the rate-limiting step during rehydration. Composition of the aqueous bulk solution and the processing conditions during powder manufacture influence the casein structure, and consequently their rehydration properties (Felix da Silva et al., 2017). In addition, the storage conditions of the powder, the particle structure and the rehydration conditions have an impact on powder solubility (Barbosa-Cánovas et al., 2005; Gaiani et al., 2009, 2006a). Crowley et al. (2015) attributed the poor solubility to the mineral depletion during DF. Powders with high protein contents produced in DF mode, show higher Ca^{2+}-activity due to the changes in milk salt equilibrium between the dispersed and the aqueous phase, which renders the powder less soluble (Baldwin, 2010; Crowley et al., 2014).

Lactose plays an important role during drying. Carbohydrates keep the native structure of dried proteins by replacing removed water molecules via hydrogen bonds (Allison et al., 1999, 1996; Prestrelski et al., 1993). Lactose prevents protein interactions as sterical spacer and by hydrogen bonding to the amino acid chain (Baldwin, 2010). According to Anema et al. (2006), caseins are considered as the main components poorly dissolving in MPC85. Mimouni et al. (2010) observed increased interactions between the casein micelles during powder storage resulting in compaction of micelles and the formation of a closely packed micelle surface layer. This is in accordance with McKenna (2000), who observed a closely packed monolayer micellar "skin" on the powder particles' surfaces too. He made cross-linking of micelles by non-micellar proteins and the close association of micelles at the surface responsible for this effect. Havea (2006) defined these as hydrophobic protein-protein interactions, which enhance the formation of poorly dispersible aggregates. Powders with a high lactose and/or a low lipid coverage on the particle surfaces showed best wetting properties (Fäldt and Bergenståhl, 1996; Gaiani et al., 2006a; Kim et al., 2002). Powder rehydration studies are commonly performed in the range of 20 - 60 °C (Anema et al., 2006; Crowley et al., 2015; Ferrer et al., 2008; Hauser and Amamcharla, 2016; Ji et al., 2016; Martin et al., 2010; Schokker et al., 2011). Higher temperatures lead to shorter rehydration times due to an increased water transfer towards the inner core of the powder particles (Anema et al., 2006; Felix da Silva et al., 2017). It should be noted that different systems are used for powder redispersion. In most of the studies a simple magnetic stirrer system was used with different filling heights and stirrer bar dimensions (Crowley et al., 2015; Kieferle et al., 2019; Lin et al., 2018; Martin et al., 2010) or overhead stirrers equipped with different stirrer blade geometries (Anema et al., 2006; Crowley et al., 2015; Havea, 2006; Mimouni et al., 2010; Schokker et al., 2011).

While this has been shown to be sufficient for standard milk powders, e.g., skim milk, for powders more difficult to redisperse, such as MPC or MC, this method is most likely not appropriate. The judgement of good or inferior rehydration behavior may therefore well depend on the mixing system used in the respective studies. Ferrer et al. (2008) found that dispersing MPC in water at 20 °C for 1 h followed by 1 h at 60 °C and a 5 min shearing with a handheld homogenizer was most effective. Nevertheless, monomodal particle size distributions (PSD) around micelle size could not be obtained for MPC containing 90% protein. It was not mentioned

in this work how the pre-shearing was conducted, but a magnetic stir bar or overhead stirrer can be assumed to have been used. Chandrapala et al. (2014) examined the effect of different shear systems on the solubility of MPC80 and MC, following a rough pre-mixing by manual shaking in a Schott bottle for 20 s. They compared the efficiency of low shear (overhead stirrer), high shear (handheld homogenizer), ultrasonication and high pressure homogenization (HPH) at the same input energy density. The authors showed that even high shear rotor stator systems (Ultraturrax) compared to low shear does not significantly downsize the PSD of MPC90 and MC. Only HPH shifted the PSD towards casein micelle size. However, the applied pressures of 120 and 200 bar were insufficient to achieve monomodally distributed particle sizes around ~0.2 μm for MPC90 and MC, respectively. It was not reported whether or not the same rheological profile of freshly produced liquid systems was achieved. In a study of Sandra and Corredig (2013) the reconstitution of MPC85 powder was mentioned in order to assess the use of this material for a study on rennetability as a function of ionic calcium level as the main purpose. Colloidal particle sizes similar to those of milk could be obtained by using a household kitchen blender for dissolving MPC85. 5 min mixing at high speed combined with heating at 66 °C for 1 min resulted in a nearly full recovery of the particle size in milk. However, as this was not the main purpose of the study, the shear conditions were not specifically defined or varied. Moreover, the principle of high speed rotating knifes like in a kitchen blender is not common for mixing or homogenizing in dairy industry.

In terms of analytical measures for powder solubility various criteria have been defined and established as routine methods: Insolubility index (International Dairy Federation (IDF) method 129A:1988), dispersability (IDF standard 87:1979) and wettability (IDF standard 87:1979). However, these criteria have been established for soluble milk powders like skim milk, whole milk or buttermilk powder. In our view, they may not be the most appropriate measures for high protein powders with poor rehydration properties.

It is also well-known that milk protein concentrates show shear-thinning behavior, which becomes more pronounced with increasing total solid contents (TS). This means that at high TS levels, the concentrate's structure rearranges to a less viscous state under shearing (Anema et al., 2014). This effect should be more pronounced if the rehydration conditions have not led to fully re-solubilized milk powders. The viscosity is relevant for e.g., pumping or stirring operations during processing. The viscosity depends on the one hand on the volume fraction (casein micelles, native and denatured serum proteins) and on the other hand on the inherent viscosity of the continuous phase (lactose-dependent) (Anema et al., 2004; Jeurnink and de Kruif, 1993; Snoeren et al., 1982). It has neither been investigated in detail so far—especially at low temperatures like 4 °C—how rehydration conditions and the resulting particle sizes are related to the rheological properties of reconstituted concentrates, nor if reconstituted concentrates show the same rheological properties as freshly produced concentrates with the same composition. Kieferle et al. (2019) reported on indications that skim milk powders diligently rehydrated overnight at 4 °C, did not show an equivalent rheological profile compared to freshly produced skim milk protein concentrates. Upon investigation of the rinsing behavior of such concentrates from UF modules it was found, however, that the shearing conditions in the UF unit significantly reduced the PSD. Obviously, despite best efforts and against expectation, the standard rehydration procedure applied prior to the rinsing experiment was not efficient enough.

Despite valuable principle insights gained from these studies on powders with different characteristics and compositions, their results can hardly be directly compared: Various low or high shear tools for redispersion were used and specific single dairy powders were determined at different temperatures, concentrations and aqueous phase compositions. To the best of our knowledge a study comparing the various prevalent milk

powders in industrial use with their different compositions and powder properties has not been conducted so far.

Therefore, the aim of this study was to compare the solubility of milk protein powders with different compositions in a controlled study under the same experimental conditions in a range covering even poorly redispersable milk protein powders. Specifically, the impact of temperature at low shear (4, 20 and 50 °C) and high pressure homogenization (50 °C, 500 bar) on the resulting rheological properties of SM, MPC50 (contains 50% (w/w) protein), MPC85 (contains 85% (w/w) protein) and MC was assessed, which also has not been studied in a direct comparison so far. As criteria, the rheological properties and particle sizes of reconstituted and fresh concentrates with same composition were compared.

This study was carried out to gain insights into the solubility or rehydration behavior of dairy powders differing in protein composition. The hypothesis was that rehydration conditions and composition of milk protein powders can lead to differences in presence of powder aggregates and thus, to variation in the resulting viscosity. Further to that we wanted to assess the effect on high pressure homogenization following the rehydration step at high pressure levels above 200 bar (Chandrapala et al., 2014) not applied for this purpose so far on the destruction of remaining powder aggregates. The PSD was measured and as a result from that, the rheological profile, which should be like their freshly produced equivalents if the powder redispersion was successful. In other words, regarding analytical measures to assess powder rehydration effectiveness, our approach was to use primary analytical measures. i.e., PSD and viscosity profile (shear stress as a function of shear rate and the power-law coefficients). This appears to be justified since the standardized routine methods are hardly applicable for highly concentrated protein powders—especially low molecular weight constituents-depleted MC and MPC.

3.1.2. Material and Methods

3.1.2.1. Milk protein powders

In this study, we used skim milk powder (SM), milk protein concentrate powder with 50% (w/w) protein (MPC50), milk protein concentrate powder with 85% (w/w) protein (MPC85) and micellar casein powder (MC). SM was supplied by Alpavit (Lauben, Germany), MPC50 by Privatmolkerei Naarmann GmbH (Neuenkirchen, Germany) and MPC85 by Baltic Dairy Board Ltd (Bauska, Latvia). Investigating reconstitution of industrial milk protein powders is of great interest, because they are commonly used for research or industrial applications. The spray drying conditions applied for the industrial powders cannot be reported in detail, since they are proprietary, but it can be assumed that they were dried in the range of standard industrial conditions. Based on the composition and degree of whey protein denaturation it is possible to draw conclusions of the processing history of the powders: For SM and MPC50 powders, the milk or ultrafiltered (UF) concentrate is usually evaporated prior spray drying to increase the total solid content. Additional diafiltration (DF) steps prior evaporation are necessary to wash out lactose and soluble salts (Na^+, K^+, Cl^-) and to reach total protein contents of 85% and higher. For MC production microfiltration (MF) combined with DF is also used to wash out whey proteins and to increase the casein concentration.

Commercial MC powders usually have casein concentrations around 90% (w/w), which is accompanied with almost complete lactose removal. For this study MC with lactose was produced to keep the casein concentration on the same level as in MPC85. Consequently, the impact of lactose combined with the impact of

casein/whey protein ratio on the solubility of high protein powders could be determined. The MC was produced as follows: pasteurized skim milk (74 °C, 28 s) of a local dairy (Molkerei Weihenstephan GmbH & Co. KG, Freising, Germany) was first twofold-concentrated from 9.73 to 13.13% total solids (TS) at 50 °C ± 2 °C using a 0.14 µm molecular weight cut-off membrane (TAMI Industries, Nyons Cedex, France). The MF permeate was collected in a second plant and UF-filtered with a 10 kDa molecular weight cut-off membrane (MICRODYN-NADIR GmbH, Wiesbaden, Germany). The resulting UF permeate was used as DF medium to keep the salt and lactose content in the serum phase constant. In 7 DF steps the whey proteins were washed out from the MF retentate. Subsequently, this was fourfold-concentrated (19.53% TS) and stored overnight at 4 °C ± 1 °C.

For spray drying, a NIRO Atomizer (GEA Group, Düsseldorf, Germany) was used. The inlet temperature was 180 °C ± 0.5 °C and the outlet temperature 80 °C ± 1 °C. The feed flow rate was 0.30 ± 0.05 °L min^{-1} and the disk rotation speed was 15.000 rpm. The powder compositions are presented in Table 3.1.

Table 3.1 Composition of the investigated dairy powders (mean ± standard deviation).

	Casein (%, w/w)	Whey protein (%, w/w)	Total protein (%, w/w)	Casein: whey protein ratio	Degree of denaturation (%)	Lactose (%, w/w)	Minerals (%, w/w)	Total solids (%)
SM	34.2 ± 0.1	5.3 ± 0.3	39.5	87:13	49.1	46.4 ± 0.9	3.9 ± 0.0	95.1 ± 0.0
MPC50	45.1 ± 0.3	6.2 ± 0.0	51.3	88:12	34.8	33.1 ± 0.2	3.5 ± 0.0	94.1 ± 0.1
MPC85	63.8 ± 0.2	10.2 ± 0.0	74.0	86:14	47.0	4.0 ± 0.0	2.3 ± 0.0	91.7 ± 0.1
MC	65.6 ± 0.5	1.4 ± 0.2	67	98:2	60.8	13.4 ± 0.2	3.5 ± 0.0	95.9 ± 0.0

Casein and whey protein content as well as the degree of whey protein denaturation (according to Dumpler et al., 2017) and lactose content (according to Schmitz-Schug, 2013) was determined by reversed-phase high performance liquid chromatography. The flame photometer ELEX 6361 (Eppendorf AG, Hamburg, Germany) was used to measure the amount of soluble minerals (Na^+, K^+) and the total Ca^{2+} concentration in the rehydrated powders.

All powders were stored in aluminum compound foil bags at 20 °C to avoid oxygen migration through the packaging material and to prevent the powder from UV radiation.

3.1.2.2. Preparation of fresh milk protein concentrates

Filtrations in lab scale were necessary to compare the rheological properties of fresh and reconstituted milk concentrates. For this, polymeric cassette membranes (Pall Corporation, New York, USA) were used with molecular weight cut-offs of 0.1 µm (MF) and 10 kDa (UF), respectively. To produce a concentrate with a composition similar to 10% (w/w) MPC50, milk was 1.5-fold UF-concentrated. For MPC85 a twofold concentration was necessary. To obtain MC, milk was microfiltered in diafiltration (DF) mode with UF permeate. After 5 DF steps the composition in terms of casein, whey protein and lactose was comparable with the 10% MC solution.

3.1.2.3. Powder rehydration and homogenization

All concentrates were adjusted to a TS content of 10% (w/w). The powders were weighed into beakers (50 mL, 38.7 mm in diameter) and filled up with demineralized water to a total mass of 20 g ± 0.1 g considering the total solid contents of the powders. To determine the impact of temperature on the particle size distribution, stirring was carried out at 4, 20 and 50 °C, respectively, with a magnetic stir bar (30.5 mm length, 6.0 mm width). Low shear was applied, so that the two influencing factors temperature and high shear could not coincide. Then, the solubility could be attributed to temperature only. Cold temperatures such as 4 °C are of interest regarding microbial growth. A common temperature to dissolve powders is room temperature (20 °C). 50 °C were applied, because it is commonly used in industry to avoid protein-protein interactions, which occur, when hydration is conducted in water at 20 °C (Havea, 2006). 50 °C decrease the viscosity and enhance the powder dissolution without negative impact on the whey proteins. Samples at 4 and 20 °C were stirred overnight, whereas samples at 50 °C were stirred for 45 min. Keeping the rehydration at high temperatures as short as possible is of interest regarding structural changes and therefore, powder functionality, energy costs and of course, microbial growth. It was not the aim to find out how long the powders need at 50 °C to be fully dissolved, but rather how the PSD looks like after a set time and temperature with or without homogenization.

Homogenization was part of the fourth experiment: In preliminary experiments all solutions were high pressure-homogenized (APV 1000, SPX Flow Technology, Crawley West Sussex, United Kingdom) between 100 and 900 bar in 100 bar steps to figure out the lowest necessary pressure to achieve a monomodal particle size distribution around casein micelle size. For the rheological measurements, the samples were homogenized at 500 bar (single stage, single path) after stirring for 45 min at 50 °C. For this, 500 g concentrate were produced as described above (800 mL beaker, 92.2 mm in diameter; stir bar with 79.3 mm length and 10.0 mm width). The pre-mixing was conducted at 50 °C, because rehydration was most effective at this temperature while keeping the whole process as short as possible.

3.1.2.4 Particle size analysis

A Malvern Mastersizer 2000 equipped with a Malvern Hydro 2000S sample dispersion unit (Malvern Instruments GmbH, Herrenberg, Germany) was used. The measurement relies on static light scattering. Particle sizes are calculated within 0.02 - 2000 µm of up to 100 sizes classes applying Mie theory (Rawle, 2003; Rawle and Kippax, 2010). This allows measuring on the one hand casein micelles and on the other hand large powder particles at once. This method is established for measuring milk powder redispersability and has been used by many studies for similar purposes (Anema et al., 2014; Bouvier et al., 2013; Chandrapala et al., 2014; Crowley et al., 2015; Ferrer et al., 2008; Ji et al., 2016; Kieferle et al., 2019; Sandra and Corredig, 2013).

The refractive indices of the dispersant (deionized water) and the protein was 1.33 and 1.41, respectively. Particle absorption index was 0.001 (according to Dumpler and Kulozik, 2016). The sample was added and dispersed at a constant stirrer speed (2000 rpm) until the obscuration reached 15% ± 1% according to the guidelines of the manufacturer. The stirring prevents the large powder particles from sedimentation. Besides the distribution density $q_3(x)$ and the cumulative distribution $Q_3(x)$, the software calculates the related $d_{10,3}$, $d_{50,3}$, and $d_{90,3}$ values, meaning 10, 50 and 90% of the particles are smaller than the respective d-value. Three different samples of each powder type were measured. Fresh skim milk, which was the reference, was measured as well. Each sample was measured in duplicate at 20 °C within 3 min.

3.1.2.5. Rheological measurements

To evaluate the influence of particle sizes on the rheological behavior of milk protein concentrates, the MCR 302 rheometer (Anton Paar GmbH, Graz, Austria) equipped with a cone/plate geometry (2°, 50 mm in diameter) was used. The sample volume was 1.15 mL and the measurement temperature 20 °C. A cone/plate geometry was chosen for this study, because it yields homogenous shear conditions for either low or highly viscous solutions. The shear rate or shear deformation in the cone gap is constant (Mezger, 2007). Furthermore, the powder particles do not have the chance to sediment during equilibration like in a cylinder.

The sample was pre-sheared at 500 s^{-1} for 1 min before resting for 15 s to allow the system to equilibrate. The shear stress was monitored over a shear ramp from 1 to 1000 s^{-1} within 9 min (according to Anema et al., 2014, modified). Each powder type was measured in duplicate from two different samples.

Flow curves were fitted with the power law model (3.1), which describes the flow behavior of a non-Newtonian fluid like milk concentrates.

$$\tau = K \cdot \dot{\gamma}^n \tag{3.1}$$

τ is the shear stress (Pa), K the consistency coefficient (Pa s^n), $\dot{\gamma}$ the shear rate (s^{-1}) and n the flow behavior index (-). If $n < 1$ the solution behaves shear-thinning, if n=1 it is Newtonian and if $n > 1$ it behaves shear-thickening. K and n depend on the total solid content of the solution: K increases and n decreases with increasing total solids (Anema et al., 2014).

3.1.2.6. Statistical analysis of data

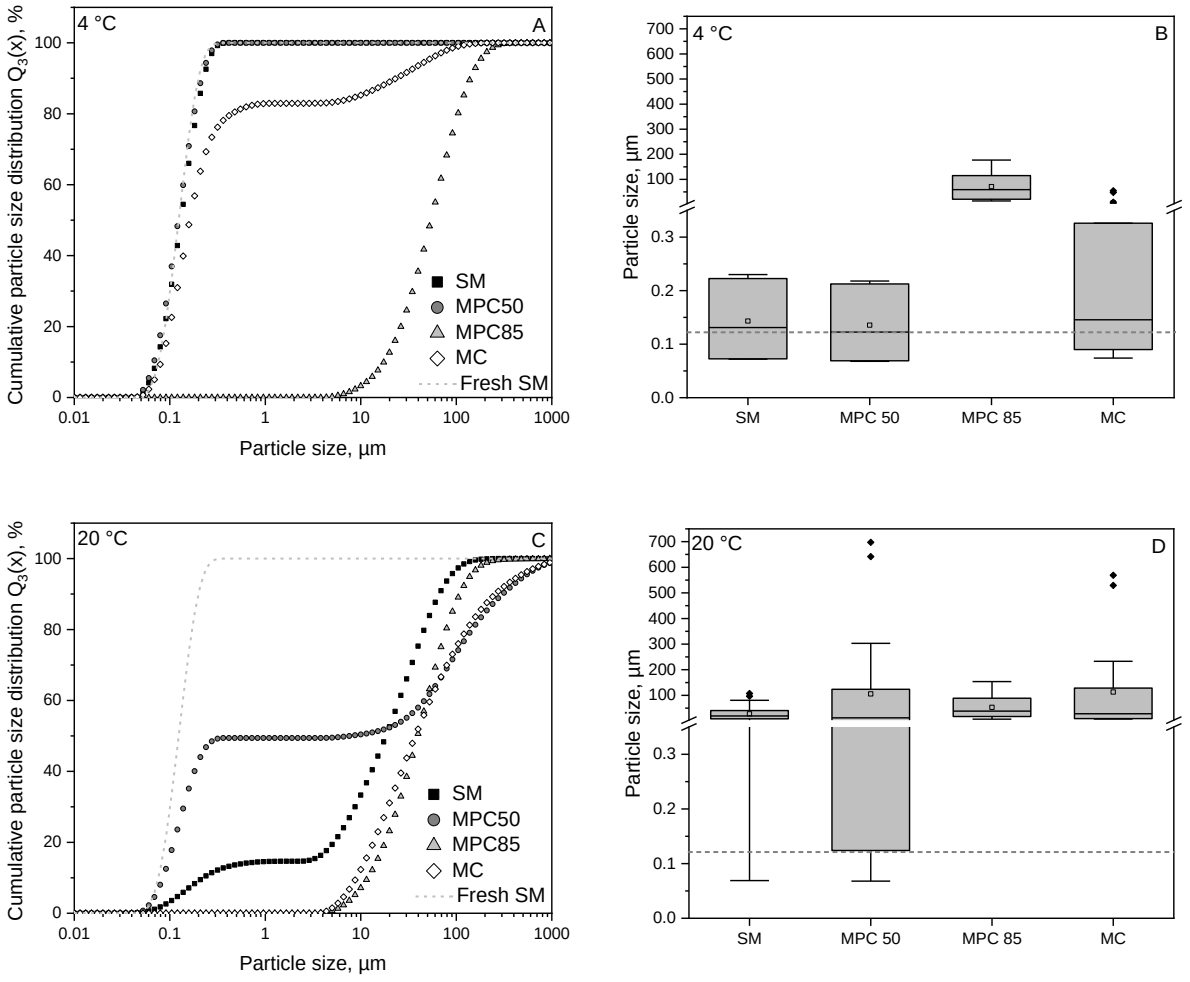
Origin 2019 (OriginLab Corporation, Northampton, United States) was used to plot graphs and to calculate the mean, the median, the median of the first and the third quartile and the outliers of the particle size distribution's $d_{10,3}$, $d_{50,3}$ and $d_{90,3}$ values illustrated as boxplots. RStudio (RStudio, Boston, United States) was used for statistical analysis. Statistical significances were evaluated using one-way analysis of variance (ANOVA) combined with Tukey's HSD post-hoc test. The calculated *P*-values are given in the text and indicate the significance level ($P \leq 0.001$, $P \leq 0.01$, $P \leq 0.05$, $P \leq 0.1$). Homogeneity of variances is proofed with Levene's test ($P > 0.05$).

3.1.3. Results and discussion

3.1.3.1. Impact of temperature on particle size and rheology

Rheological measurements were performed to investigate the relation between particle size and the rheological properties of a reconstituted concentrate.

Figure 3.1 shows the cumulative particle size distribution (PSD) and the $d_{10,3}$, $d_{50,3}$ and $d_{90,3}$ values illustrated as boxplots for each powder at all temperatures.



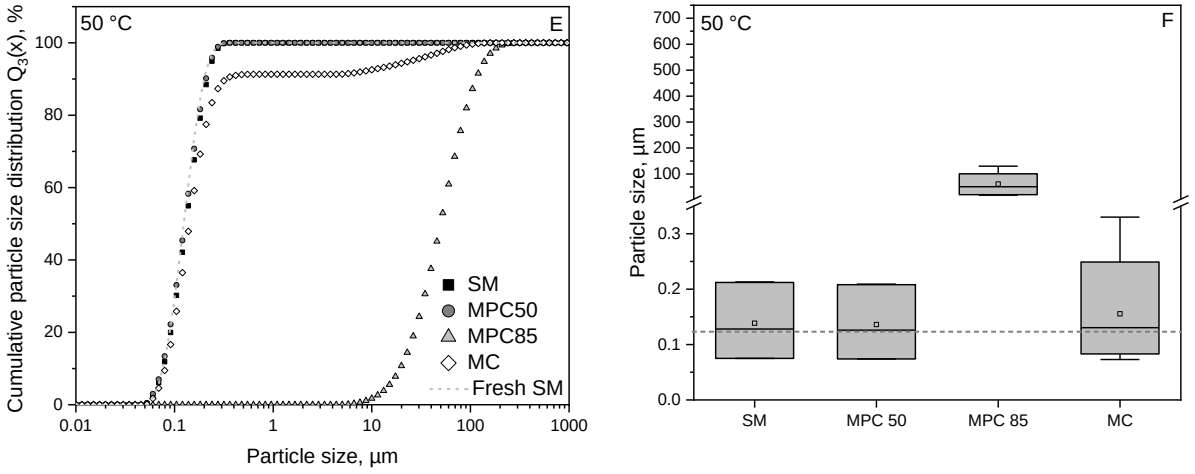


Figure 3.1 Cumulative particle size distribution $Q_3(x)$ (volume % vs. diameter) (left) and particle size distribution presented as boxplots (right) of SM, MPC50, MPC85 and MC at 4 °C (A, B), 20 °C (C, D) and 50 °C (E, F). Boxplots: The low and high quartiles, the median and the mean (□) are plotted. (◆) represents outliers, (- - -) the $d_{50.3}$ of fresh skim milk.

At 4 °C skim milk powder (SM) and milk protein concentrate powder with 50% (w/w) protein (MPC50) were dissolved completely (Figure 3.1 A, B). Their mean was around the casein micelle size (~150-200 nm) and their $d_{50.3}$ corresponded to the $d_{50.3}$ of fresh skim milk (0.124 µm). The particle sizes of micellar casein powder (MC) were poorly reproducible, which explains the extended size of the boxplot. In one experiment it was completely dissolved, in another it contained large powder particles. Milk protein concentrate powder containing 85% protein (MCP85) showed the worst rehydration properties: Its mean was around 60 µm indicating remaining large powder particles. Hauser and Amamcharla (2016) improved the solubility of MPC by raising the dissolution temperature from 40 to 48 °C.

Increasing the temperature here from 4 to 20 °C did not improve the rehydration properties of any powder, rather making it worse. All solutions still contained undissolved powder particles (Figure 3.1 C, D). Even SM showed poor rehydration characteristics at 20 °C. The particle sizes of SM, MPC50 and MC increased significantly compared to 4 °C ($P \leq 0.05$, $P \leq 0.1$ and $P \leq 0.01$, respectively). The amount of outliers as well as the sizes of the boxplots indicate a low reproducibility. This implies that 20 °C is not a good choice as powder rehydration temperature if reproducible product characteristics are desired. Higher temperatures are said to lead to shorter total rehydration times (Felix da Silva et al., 2017). For the temperature increase from 4 to 20 °C this could not be verified.

Therefore, the temperature was further increased and the powders were dissolved at 50 °C (Figure 3.1 E, F). This led to significantly smaller particle sizes for SM, MPC50 and MC ($P \leq 0.05$, $P \leq 0.05$ and $P \leq 0.001$, respectively). SM and MPC50 were already completely, and MC almost completely dissolved after 45 min. Only MPC85 still contained particles around 60 µm as at 4 and 20 °C. This is in accordance with literature concerning poor solubility of MPC85 at room temperature (Gaiani et al., 2006b), 22 °C (Crowley et al., 2015), 30 °C (Anema et al., 2006) and 48 °C (Hauser and Amamcharla, 2016). It has not been reported before that low temperatures like 4 °C do neither enhance nor impair the solubility of MPC85 compared to ambient or warm temperatures.

The target was to achieve the monomodal PSD of fresh skim milk. In fresh skim milk casein micelles have a particle size range between 0.1 and 0.2 μm and a $d_{50,3}$ of 0.124 μm. Stirring at 20 °C led to monomodal PSD around 10 μm and larger for all powders (Figure 3.1 C). At 4 °C and 50 °C SM and MPC50 were monomodally distributed around 0.14 μm, MC bimodal around 0.2 and 50 μm and MPC85 remained as poorly rehydrated as at 20 °C (Figure 3.1 A, E).

Apart from the influence of dissolving temperature on the PSD, the impact of particle size on the rheology of reconstituted skim milk concentrates was investigated. The flow behavior of all concentrates were measured at 20 °C. Figure 3.2 shows the shear stress as a function of shear rate for all concentrates after stirring at 4, 20 and 50 °C.

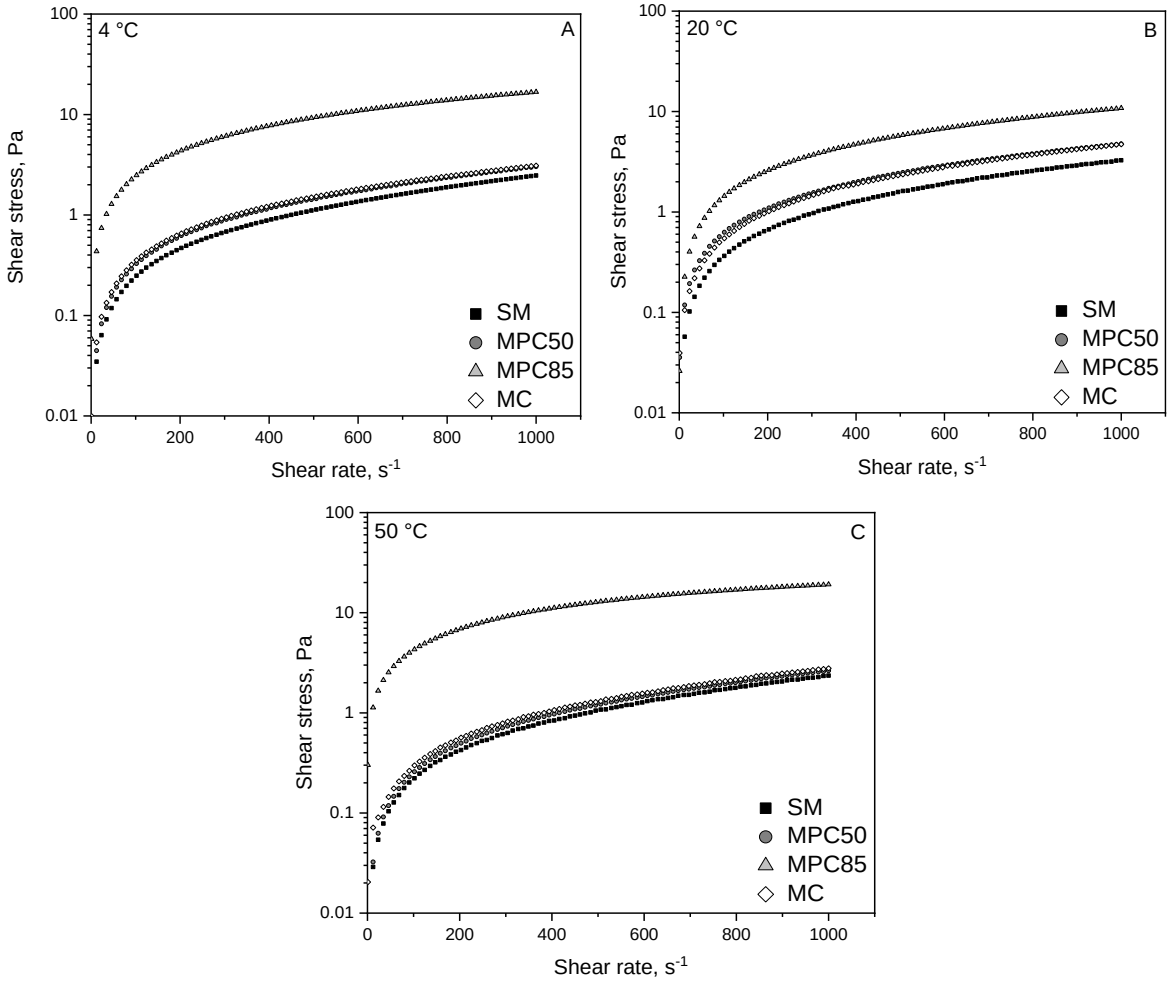


Figure 3.2 Shear stress as a function of shear rate of SM, MPC50, MPC85 and MC after stirring at 4 °C (A), 20 °C (B) and 50 °C (C).

Apart from that, the powder rehydration properties are strongly dependent on the presence or absence of fast dissolving components (FDC) such as lactose and whey proteins. Caseins are considered as slow dissolving components. The latter are rate limiting in MPC rehydration (Mimouni et al., 2010).

The powders used here can be sorted by their amounts of fast dissolving components: MPC85 < MC < MPC50 < SM. Lactose is hydrophilic and acts as pathway for moisture transfer into the casein micelle during

rehydration (Baldwin, 2010). The less lactose the powder contains, the slower is the water penetration into the powder particles and the worse are the results of the dissolution behavior. The insignificantly ($P \leq 0.1$) different particle size results of 4 and 50 °C (Figure 3.1) indicate that these temperatures support the release of FDC and the dissolution of casein micelles in SM, MPC50 and partly MC. Low temperatures are known to improve water absorption into the casein micelle and the release of calcium phosphate and β-casein, which leads to a partial loss in micellar integrity and, thus, to a more porous structure (Gaucheron, 2005). This might be the reason for the better solubility of the powders—except from MPC85—at 4 °C compared to 20 °C. This seems to be an interesting observation since ambient temperatures have often been used as standard rehydration temperature in other studies. Higher temperatures such as 50 °C decrease the viscosity and enhance the molecular mobility. Under these conditions water can migrate faster into the powder particles, and even slow dissolving casein micelles in SM and MPC50 fully rehydrate within 45 min and in MC to the maximal extent possible given under these stirring conditions.

At 4 °C SM showed the lowest shear stress across the whole shear rate range, whereas MPC50's and MC's were slightly higher. MPC85 differed significantly from those values ($P \leq 0.001$). This was also reflected in the consistency coefficient K and the flow behavior index n (Table 3.2) indicating the highest consistency index of MPC85 with most pronounced non-Newtonian shear-thinning behavior. This implies that shear-thinning does not only depend on the total solid content, but also on the particle sizes in the solution. The larger the particles the stronger the shear-thinning behavior.

A temperature increase from 4 to 20 °C led to significantly higher shear stresses for SM, MPC50 and MC ($P \leq 0.001$) with higher K and lower n-values due to the increased particle sizes. SM's shear stress increased by 0.8 Pa at 1000 s^{-1}, MPC50's and MC's by 1.62 Pa. In contrast to that, the shear stress of MPC85 significantly decreased by 5.95 Pa ($P \leq 0.001$). This can be attributed to its lower mean particle size at 20 °C (50.57 μm) compared to 4 °C (70.96 μm). As a result of that, the solution shows a less pronounced shear-thinning behavior.

Also, the PSD at 50 °C was directly reflected in the shear stress: SM, MPC50 and MC had particle size means around micelle size, which led to a shear stress decrease to 2.36 Pa (SM) and 2.79 Pa (MPC50 and MC) at 1000 s^{-1}, a lower consistency index combined with a more Newtonian behavior. The mean of MPC85's PSD was still 60.99 μm, resulting in higher consistency coefficient and the significantly higher shear stress of 19.16 Pa at 1000 s^{-1} ($P \leq 0.001$). The shear stresses as well as the consistency coefficient of SM, MPC50 and MC decreased significantly ($P \leq 0.001$) when the temperature was increased from 20 to 50 °C, whereas K and the shear stresses at 4 compared to 50 °C did not differ significantly ($P \leq 0.1$).

Table 3.2 Calculated consistency coefficient K and flow behavior index n (mean ± standard deviation). Correlation coefficient R^2 indicates how well the power law model fits the measured data. P-values are given to prove homogeneity of variance for the groups [a,b,c].

		Consistency coefficient K [mPa s^n]	Flow behavior index n [-]	Correlation coefficient R^2 [-]
4 °C	SM	3.68 ± 0.6	0.93 ± 0.0	0.98
	MPC50	3.60 ± 0.4	0.97 ± 0.0	1.00
	MPC85	54.66 ± 7.8	0.83 ± 0.0	1.00
	MC	6.14 ± 0.8	0.89 ± 0.0	0.99
20 °C	SM	6.01 ± 1.4	0.90 ± 0.0	0.99
	MPC50	3.96 ± 0.4	0.94 ± 0.2	0.99
	MPC85	25.24 ± 3.2	0.87 ± 0.0	1.00
	MC	13.96 ± 2.1	0.83 ± 0.0	0.98
50 °C	SM	2.97 ± 0.1	0.95 ± 0.0	0.98
	MPC50	3.20 ± 0.1	0.96 ± 0.0	0.99
	MPC85	235.68 ± 10.9	0.64 ± 0.0	1.00
	MC	7.07 ± 2.6	0.85 ± 0.1	0.97
Homogenized	SM	2.66 ± 0.5	0.98 ± 0.0	0.99
	MPC50	2.62 ± 0.9	1.00 ± 0.1	0.99
	MPC85	4.66 ± 0.7	0.95 ± 0.0	1.00
	MC	3.33 ± 0.1	0.94 ± 0.0	0.99
Fresh	SM	2.04 ± 0.4	1.01 ± 0.0	0.99
	MPC50	3.24 ± 0.4	0.96 ± 0.0	0.99
	MPC85	5.83 ± 1.7	0.94 ± 0.0	1.00
	MC	2.93 ± 0.2	0.96 ± 0.0	0.99

[a] Consistency coefficient K: $P = 0.5196$, flow behavior index n: $P = 0.3326$

[b] Consistency coefficient K: $P = 0.3456$, flow behavior index n: $P = 0.1668$

[c] Consistency coefficient K: $P = 0.6339$, flow behavior index n: $P = 0.9180$

The results show that the powder composition influences the PSD of the concentrate and this in turn, determines its rheological properties. The larger the particles, the stronger was the shear-thinning behavior. In general, the viscosity depends on the volume fraction of the contributing particles as well as on the inherent viscosity of the continuous phase. The lactose concentration determines the viscosity of the continuous phase, whereas the proteins contribute to the volume fraction; implying casein micelles, dissociated caseins, native and denatured whey proteins (Anema et al., 2004; Jeurnink and de Kruif, 1993; Snoeren et al., 1982). Casein micelles largely contribute to the viscosity of skim milk due to their relatively high concentration and numerous interactions (Walstra et al., 1984). During concentration in MPC or MC production, water containing lactose and salt is removed. The distance between casein micelles decreases; therefore, they interact more intensely, resulting in a higher apparent viscosity and a more shear-thinning behavior (de Kruif, 1997; Karlsson et al., 2005). This can lead to reduced flow rates, high pressure drops, decreased turbulence and severe fouling in heating operations during milk processing (Bienvenue et al., 2003b).

3.1.3.2. Impact of high pressure homogenization on particle size and rheology

In a further experiment the impact of high pressure homogenization (HPH) on the particle size and the rheology of reconstituted skim milk concentrates was determined.

In preliminary experiments all concentrates were homogenized from 100-900 bar in 100 bar steps to figure out the lowest necessary pressure to achieve a monomodal distribution around micelle size. Pressure did not change the PSD of SM and MPC50—it was already monomodal after 45 min at 50 °C with a mean particle size of 0.14 µm (Figure 3.3 A, B). MPC85 showed a monomodal distribution already after homogenization at 200 bar (Figure 3.3 C), whereas MC required 500 bar to be fully dissolved (Figure 3.3 D).

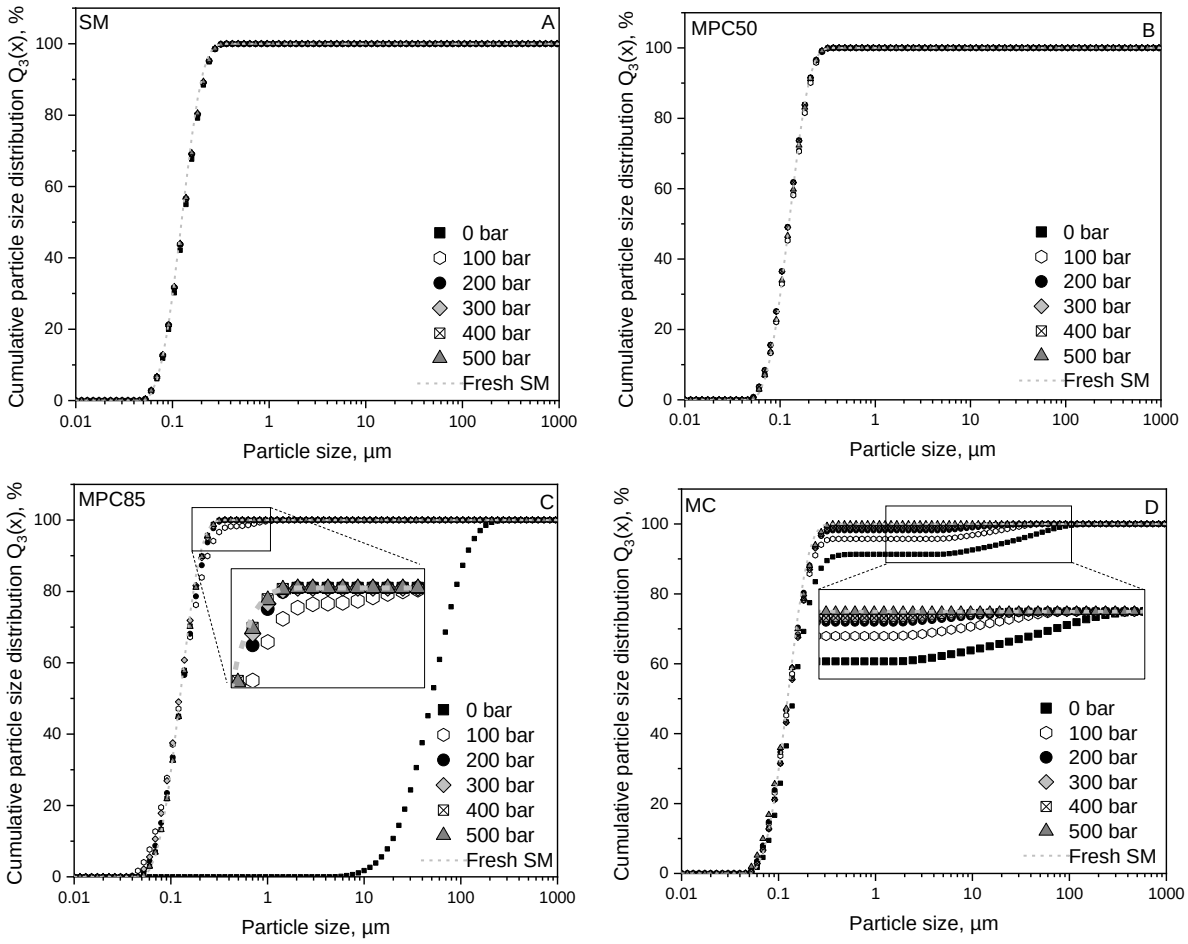


Figure 3.3 Cumulative particle size distribution $Q_3(x)$ (volume % vs. diameter) of SM (A), MPC50 (B), MPC85 (C) and MC (D) after stirring at 50 °C (0 bar) followed by HPH at 100, 200, 300, 400, and 500 bar. (- - -) represents the PSD of fresh skim milk.

The shear conditions, such as shear rate, cavitation and turbulent flow are involved in particle destruction in the HPH. The higher the pressure, the higher the gap velocity and therefore, the shear rate. Cavitation intensity increases with increasing homogenization pressure (Håkansson, 2015). Interestingly, MC contained as much casein as MPC85, more fast dissolving components, but required higher pressure to achieve a monomodal PSD. However, the ratio of whey proteins to lactose was different: MPC85 contained more whey proteins and less lactose. For MC the opposite was found. This leads to the assumption that lactose is the fast

dissolving component, which is more decisive regarding powder solubility than the whey proteins. But the less whey proteins the powder contains, the more the casein micelles interact and the more pressure—and consequently a higher shear rate and more intense cavitation—must be applied to completely dissolve the powder. Another investigated MC powder containing 83.3% casein, 4.7% whey protein and only 5% lactose needed 900 bar to be completely dispersed (data not shown). Such rehydration conditions, to the best of our knowledge, have not been reported as required for a full redispersion of milk protein powders, which are known to be poorly dissolvable.

Therefore, all concentrates were homogenized at 500 bar to establish the same initial conditions. The particle sizes of the homogenized concentrates are shown in Figure 3.4 A and B.

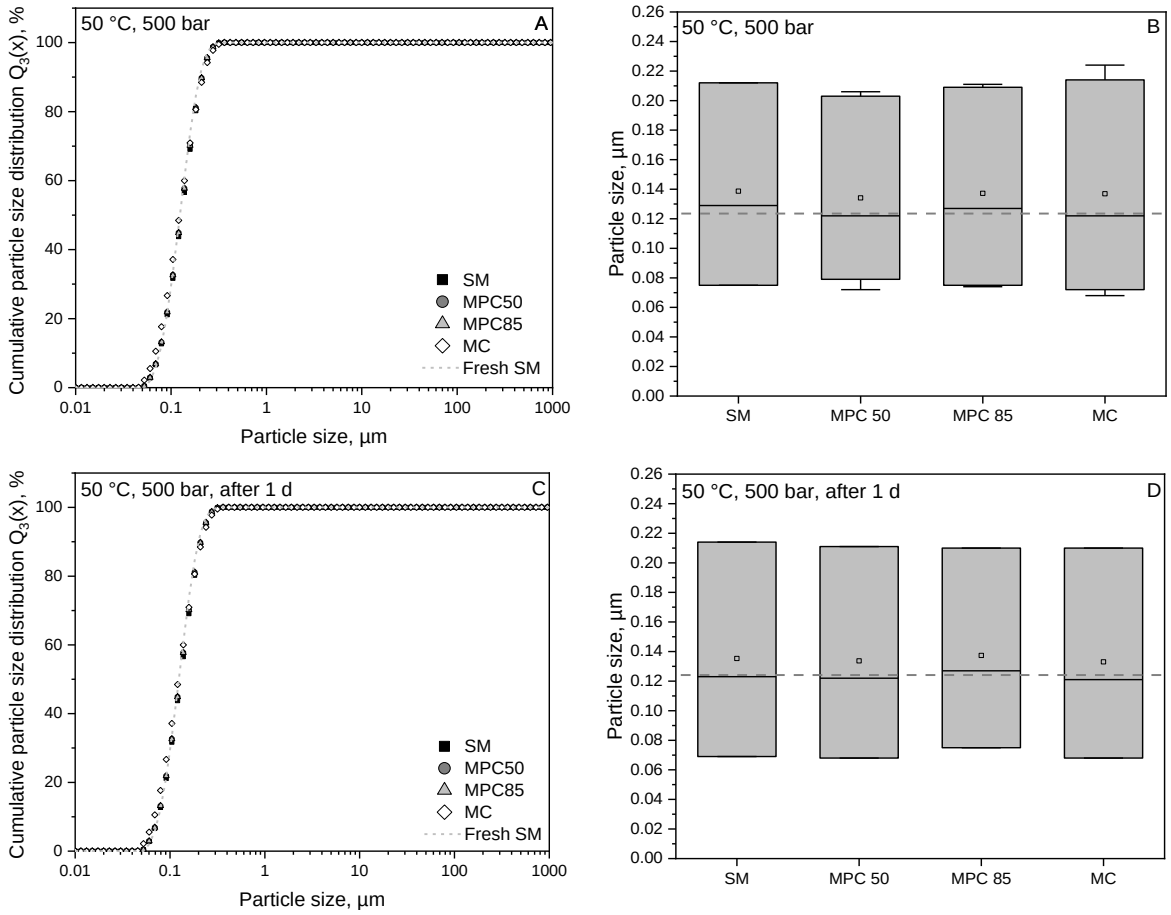


Figure 3.4 Cumulative particle size distribution $Q_3(x)$ (volume % vs. diameter) (left) and particle size distribution presented as boxplots (right) of SM, MPC50, MPC85 and MC after stirring at 50 °C followed by HPH at 500 bar (A, C) and after 1 d stored at 4 °C (B, D). Boxplots: The low and high quartiles, the median and the mean (□) are plotted. (- - -) represents the $d_{50,3}$ of fresh skim milk.

HPH lead to a mean particle size of 0.13 µm—and therefore micelle size—for all concentrates. Especially the particle sizes of MPC85 decreased significantly ($P \leq 0.001$). The results show that each of these dairy powder types must be pre-treated differently depending on the powder composition. The lower the amount of fast dissolving components the slower the dissolution of the powder particles in water—independent from temperature. This makes an additional homogenization step essential.

Even though it is not obviously reflected in the viscosity, powder particles may still remain with potentially negative implications for MPC or MC functionality (e.g., gelling or emulsification) (Crowley et al., 2015). Karam et al. (2016) found that the textural properties of acid gels obtained from reconstituted, heated (95 °C/ 5 min) SM (10% w/w) enriched with 2% (w/w) MC strongly depend on the rehydration duration (5, 120, 240 or 1440 min). For instance, the shorter the rehydration time, the softer the gel and the higher the graininess and syneresis. The authors mentioned the slow dissolution of MC, but they did not discuss their findings. It can be assumed that remaining powder particles are the reason for the differences in physical properties.

Another important question is whether HPH leads to implications in casein micelle's integrity and functionality. Sandra and Dalgleish (2007) observed the loss of some surface κ-casein on the micelle's surface due to ultra-high pressure homogenization at 1790 bar for 6 passes, which led to a slightly reduced rennet coagulation time. Lodaite et al. (2009) investigated the rheological properties (rennet gel formation) of HPH-treated skim milk. They showed that HPH at 1000 bar (55 °C, 50 bar back pressure) did neither affect the casein micelle size nor the rennet gelation properties (gel formation time, gel formation rate and storage modulus after 2700 s). Sandra and Dalgleish (2005) observed a decreased turbidity and micelle size with increasing HPH passes (up to 6) at 1860 bar. They interpreted these results with partial disintegration of the micelles, which was also reported by Needs et al. (2000) and Regnault et al. (2004) at isostatic high pressures of 2000 bar. High pressure mainly disrupts ionic and hydrophobic interactions as reported by Datta and Deeth (1999), which are known to be the stabilizing forces in casein micelle's structure (Holt and Horne, 1996; Horne, 2002; Walstra, 1999). This combined with cavitation, turbulence and shear effects during ultra-high pressure homogenization may cause the micelle disintegration (Sandra and Dalgleish, 2005). Interestingly, Lodaite et al. (2009) observed a decreased micelle size with increasing HPH pressure (2000-3000 bar) as well, but analysis of the supernatant proved only α_{S1}-casein dissociation from the micelles. Its amount in the supernatant was highest at 2000 bar. The exact mechanism of this release is still unknown. Nevertheless, the gelation properties were altered with decreasing micelle size (shorter gelation time, higher gel formation rate and higher storage modulus after 2700 s). Hernández and Harte (2008) found that HPH in combination with heat (90 °C, 5 min) improved the rheological properties and stability of yogurt, which offers the possibility to reduce additives.

There are multiple studies dealing with the impact of HPH on the casein micelle structure, but fresh milk or already dispersed reconstituted milk were used. Information lacks regarding powder reconstitution with HPH and its impact on the casein's structure. Chandrapala et al. (2012) could show that using ultrasound (20 kHz, 450 W, 50% amplitude) for 1 h disrupt casein and casein/whey protein aggregates in MPC without affecting the native state of the casein micelles. Since modifications of casein micelles' size and structure were observed at pressures higher than 1000 bar only, we can conclude that casein micelle's integrity and functionality is not impaired by HPH treatment at 500 bar.

For analytical measurements protein solutions are often stored overnight to give the proteins time to "fully hydrate" after rehydration. To evaluate, if this process is fully achieved by HPH, the PSD was measured again after 1 d of storage at 4 °C without stirring. It turned out that the PSD of all solutions did not significantly change ($P \leq 0.001$) overnight (Figure 3.4 C, D). Hence, it can be assumed that powder particle agglomerates have been completely separated and the proteins in all solutions were already fully hydrated after HPH. This enables analytical measurements of high protein powder solutions directly after rehydration with HPH within ~50 min.

The impact of homogenization on the rheological properties is shown in Figure 3.5.

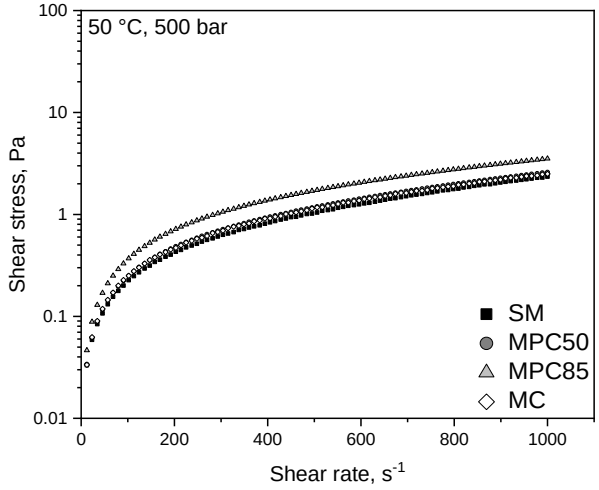


Figure 3.5 Shear stress as a function of shear rate of SM, MPC50, MPC85 and MC after stirring at 50 °C followed by HPH at 500 bar.

Homogenized MPC50, MC and SM behaved equally: all showed a shear stress of 2.50 Pa at 1000 s^{-1} and almost Newtonian flow behavior indicating the high flow behavior index *n* (Table 3.2). The shear stress of MPC50 and MC as well as the consistency coefficient *K* slightly decreased compared to the non-homogenized samples (50 °C). The shear stress of MPC85 decreased significantly from 19.16 Pa to 3.53 Pa at 1000 s^{-1} ($P \leq 0.001$) and the fluid became more Newtonian. Song et al. (2013) reported similar observations for reconstituted soy protein isolate. The viscosity of the high pressure-homogenized sample (2070 bar) was lower than those of the untreated reference. They explained the viscosity decrease by the reduced PSD after HPH and the resulting higher mobility of particles in the bulk phase.

It is known that with increasing dry matter the apparent viscosity of milk increases exponentially (Snoeren et al., 1982). A similar behavior was observed for the consistency coefficient by Anema et al. (2014).The still significantly higher shear stress and consistency coefficient of MPC85 ($P \leq 0.001$) is attributed to the highest volume fraction the powder contains compared to the other milk powder systems, combined with the lowest lactose amount (Anema et al., 2004; Jeurnink and de Kruif, 1993; Snoeren et al., 1982). Hence, the shear stress and consistency was highest—with or without HPH. Since all concentrates showed similar particle sizes after HPH, our results show that even at the same dry matter and PSD, the composition of the powder determines the viscosity.

3.1.3.3. Flow behavior comparison of fresh and reconstituted skim milk concentrates

Finally, the question was, especially with regard to comparing results from scientific studies, whether reconstituted and fresh concentrates with similar composition show the same flow behavior—when pre-treated appropriately—and if fresh concentrates can be substituted by reconstituted concentrates. Figure 3.6 shows the shear stresses as a function of shear rate for freshly produced, non-homogenized (dissolved at 20 °C) and homogenized SM, MPC50, MPC85 and MC concentrates.

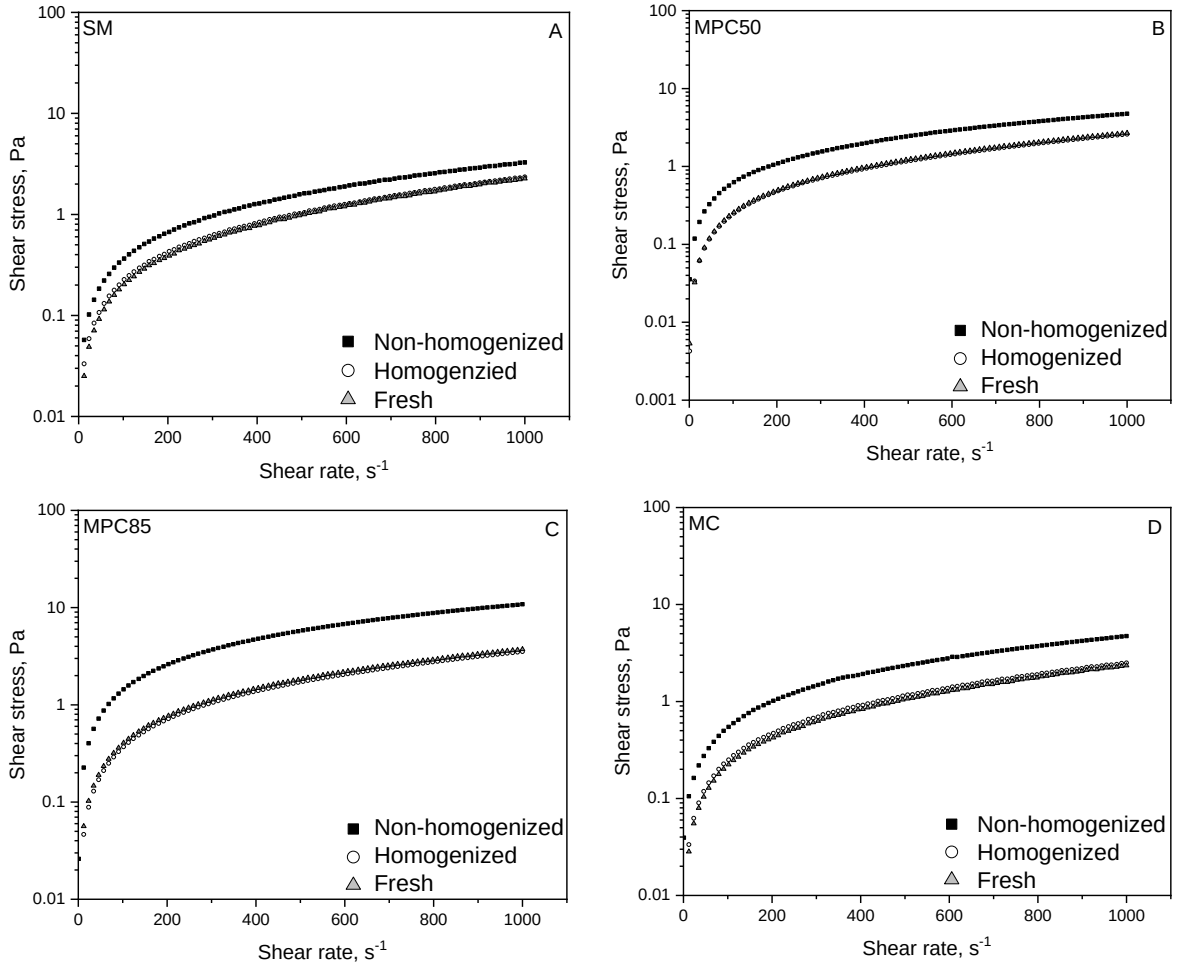


Figure 3.6 Shear stress as a function of shear rate of reconstituted and fresh SM (A), MPC50 (B), MPC85 (C) and MC (D).

Homogenization led to significantly lower shear stresses for all powders compared to non-homogenized ones ($P \leq 0.001$).

For each powder homogenization resulted in comparable flow indices as the related freshly produced concentrate (Table 3.2). It should be noted that there was no significant difference between the shear stress, consistency coefficient K and flow behavior index n of the homogenized and at 50 °C stirred samples of SM and MPC50 ($P \leq 0.1$). Hence, this treatment was sufficient to achieve the viscosities of fresh concentrates. Nevertheless, for MPC85 and MC the HPH step was essential. Also freshly produced MPC85 showed the highest shear stress and consistency compared to the other fresh concentrates. Sauer et al. (2012) showed as well that a reduction in serum protein and lactose resulted in a viscosity increase of fresh MC due to the relatively

higher volume fraction. The higher the amount of soluble components (serum proteins, lactose and minerals) and the lower the casein concentration, the lower the casein-casein interactions and hence, the viscosity.

3.1.4. Conclusions

In this study we investigated the impact of temperature (4, 20 and 50 °C) and high pressure homogenization (HPH) (500 bar) on the rehydration and rheological properties of skim milk powder (SM), milk protein concentrate powder with 50% protein (MPC50) and 85% protein (MPC85) and micellar casein powder (MC). As assessment criteria, the rheological properties of reconstituted and fresh concentrates were measured.

Applying HPH up to 500 bar on poorly reconstitutable dairy powder suspensions—especially those with a low content of fast dissolving components—appears to be indispensable for fully dissolving highly concentrated protein powders. We could confirm our hypothesis that remaining powder aggregates increase the viscosity of reconstituted skim milk concentrates compared to freshly produced ones with the same composition. Our results show that each powder type—depending on the composition—requires a different shear treatment to achieve the same particle size distribution like fresh skim milk and the same flow behavior of equivalent fresh concentrates. Low protein powders like SM or MPC50 dissolve completely under low shear at 4 °C overnight or at 50 °C within 45 min. MPC85 and MC needed an additional HPH step to disrupt all remaining powder particles. If the rehydration conditions are adapted to the respective powder type, fresh concentrates are completely substitutable with reconstituted concentrates. Further work should validate these results on the rehydration conditions required for redispersing different milk protein powders in amounts typical for industrial applications. Using HPH for powder redispersion in the industry is cost-effective because this technology is already used commonly in dairies for milk homogenization and can be applied for this purpose too.

Acknowledgments: The authors wish to thank Claudia Hengst, Heidi Wohlschläger, Hermine Roßgoderer and Martin Hilz for help in conducting the analytical measurements. Special thanks go to Daniela Panoch for implementing experiments; to Mine Özcelik, Sabine Ambros, Michael Reitmaier and Roland Schopf for their support and valuable discussions.

3.2. *Functionality of MC88- and MPC85-enriched skim milk: Impact of shear conditions in rotor/stator systems and high-pressure homogenizers on powder solubility and rennet gelation behavior*

Summary and contribution of the doctoral candidate

Milk protein concentrate (MPC) and micellar casein (MC) powders are commonly used to increase the protein concentration of cheese milk. However, highly-concentrated milk protein powders are challenging in terms of solubility. The research question was whether and how incompletely dissolved agglomerates affect the protein functionality in terms of rennet gelation behavior.

To evaluate the impact of remaining powder aggregates on the protein functionality, the rennet gelation behavior of the MC88- and MPC85-enriched skim milk was investigated. For the experiments, skim milk was enriched with either MC88 or MPC85 to a casein concentration of 4.5% (w/w). The assessment criteria were particle size as a function of shear rate and the rennet gelation properties. As for cheese manufacture, gelling time, gel strength, structure loss upon deformation, and serum loss were measured. Furthermore, the casein, whey protein, and casein macropeptide (CMP) recovery in the sweet whey was determined to evaluate the shear- and hence, particle size-dependent protein accessibility.

In the dairy industry, two high shear units are widely implemented and can be used for powder redispersion: shear pumps like in powder mixers and HPH. Therefore, we performed experiments on a laboratory scale first, using a colloid mill as rotor/stator system and a laboratory scale HPH. For the subsequent upscaling experiments a shear pump and a pilot scale HPH were used.

It turned out that applying HPH (100 bar) on poorly soluble dairy powders like MC88 and MPC85 containing high ratios of slow dissolving components appears to be indispensable and sufficient for full powder dissolution. The flow conditions in the rotor/stator systems were insufficient—even at shear rates up to 7.4×10^4 s^{-1}—for complete powder destruction; additional cavitation, which occurs in HPH, was required.

It could be shown that insufficient powder rehydration prolongs the rennet gelation time. Although the gelation time of MC88- and MPC85-enriched skim milk was similar, the addition of MPC85 resulted in softer, weaker gels and consequently, in a reduced syneresis/serum loss. Moreover, remaining powder particles decrease the whey protein concentration in the sweet whey. It was concluded that remaining powder particles impair the rennet gelation behavior due to the inaccessible proteins in the powder aggregates as well as the gel properties, which was disturbed by large powder particles.

The most significant contribution to this manuscript was made by the doctoral candidate. This comprised the conception and design of experiments based on preceded critical literature review as well as major conduction of data analysis, data interpretation, and discussion. In addition, writing and editing of the manuscript was done by the doctoral candidate. The co-author contributed to the project outline, the discussion of results, and the revision of the manuscript.

Adapted original manuscript[3]

Functionality of MC88- and MPC85-enriched skim milk: Impact of shear conditions in rotor/stator systems and high-pressure homogenizers on powder solubility and rennet gelation behavior[4]

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Abstract: Milk protein concentrate (MPC) and micellar casein (MC) powders are commonly used to increase the protein concentration of cheese milk. However, highly-concentrated milk protein powders are challenging in terms of solubility. The research question was whether and how incompletely dissolved agglomerates affect the protein functionality in terms of rennet gelation behavior. For the experiments, skim milk was enriched with either MC88 or MPC85 to a casein concentration of 4.5% (w/w) and sheared on a laboratory and pilot scale in rotor/stator systems (colloid mill and shear pump, respectively) and high-pressure homogenizers. The assessment criteria were on the one hand particle sizes as a function of shear rate, and on the other hand, the rennet gelation properties meaning gelling time, gel strength, structure loss upon deformation, and serum loss. Furthermore, the casein, whey protein, and casein macropeptide (CMP) recovery in the sweet whey was determined to evaluate the shear- and hence, the particle size-dependent protein accessibility. We could show that insufficient powder rehydration prolongs the rennet gelation time, leads to softer, weaker gels, and to lower amounts of CMP and whey protein in the sweet whey.

Keywords: Cheese manufacture; Milk protein powders; Rehydration; Dissolution; Particle size; Shear rate; Powder aggregates; Protein accessibility; Upscaling

3.2.1. Introduction

In cheese manufacture, it is common to increase the casein or the total protein concentration of the vat milk to increase the cheese yield (Anema et al., 2006; Singh, 2007). There are two possibilities to vary the total protein concentration or the casein/whey protein ratio: First, by micro- or ultrafiltration or second, by adding high protein powders like micellar casein (MC) or milk protein concentrate (MPC) powders (Guinee et al., 2006). With powders the protein content can be standardized flexibly, and seasonal fluctuations of the milk composition can be compensated (Guinee et al., 2006). These are known to have a major impact on the curd forming properties and, on the composition and the yield of the final cheese (Auldist et al., 1996; Banks and Tamime, 1987; O'Brien et al., 1999; O'Keeffe, 1984). However, the redispersion of high protein powders can be

[3] Adaptions refer to formatting issues: e.g., numbering of sections, figures, tables, and equations; abbreviations, manufacturer specifications, axis labeling, figure captions, and style of citation. References are listed at the end of this thesis, combined with the references of the other publications, to avoid redundancies.

[4] Originally published in: Foods (2021), 10(6), 1361.

challenging in terms of solubility due to their insufficient dissolving ability (Baldwin and Truong, 2007), which is often neglected in studies assuming that rehydration overnight would ensure full powder dissolution.

According to Oldfield and Singh (2005), the extent of protein interactions that occur upon pre-heating before evaporation and spray drying in milk powder production affects the powder solubility and shelf life. Crowley et al. (2015) attributed the poor solubility of MPC to the mineral depletion during diafiltration. For high protein powder production, milk protein concentrates are usually diafiltered with water to wash out lactose and salts and to increase the protein concentration. Consequently, the powder shows a higher Ca^{2+}-activity due to changes in the milk salt equilibrium between the aqueous and dispersed phase, which renders the powder less soluble (Baldwin, 2010; Crowley et al., 2014). In a previous study we could already show that even poorly dissolvable MC and MPC85 were fully solubilized with high-pressure homogenization (HPH) resulting in the particle size distribution (PSD) of fresh skim milk (Warncke and Kulozik, 2020). Furthermore, we found out that remaining powder particles increase the shear stress of reconstituted milk protein concentrates. However, it was not investigated so far whether and how incompletely dissolved powder agglomerates affect the protein functionality in terms of rennet gelation behavior.

Ferrer et al. (2008) investigated MPC56, MPC70, and MPC90 regarding their renneting properties. The authors reported that the gel strength of MPC90 was lower after 3 h oscillating in the linear viscoelastic region (LVR) compared to MPC56 and MPC70. This is not surprising considering the particle sizes of the powders, which were 0.14 μm in MPC56 and MPC70 samples compared to 0.92 μm in the MPC90 sample. Martin et al. (2007) observed an increased strength of rennet gels made of reconstituted milk powders in water with increasing reconstitution time. Upon adding at least 1 mM calcium chloride the gel strength massively increased with increasing calcium chloride concentration. This led the authors to the assumption that some calcium in the environment (added in pure form or released from the powder particles) is necessary to induce aggregation. They suspected that the incubation time might have been too short to achieve full calcium release from the powder particles resulting in softer gels. This can also be explained with insufficient powder rehydration; it can be assumed that calcium may dissolve faster the better the powder particles were rehydrated. All three studies performed the powder dissolution on a laboratory scale. We wanted to investigate whether the shear impact on a laboratory scale is comparable to that on a pilot scale when applying the same shear rates. The transferability to industrial scale, however, remains to be demonstrated.

Many different low and high shear systems can be found the literature for powder redispersion in laboratory scale: magnetic stir bars (Crowley et al., 2015; Kieferle et al., 2019; Lin et al., 2018; Martin et al., 2010), overhead stirrers equipped with different stirrer blade geometries (Anema et al., 2006; Crowley et al., 2015; Havea, 2006; Mimouni et al., 2010; Schokker et al., 2011), handheld homogenizers (Chandrapala et al., 2014; Ferrer et al., 2008), ultrasonication (Chandrapala et al., 2014), as well as HPH (Chandrapala et al., 2014; Warncke and Kulozik, 2020). Chandrapala et al. (2014) examined the effect of different shear systems on the solubility of MPC80 and MC. They compared the efficiency of low shear (overhead stirrer), high shear (handheld homogenizer), ultrasonication, and HPH not at the same shear rate but at the same input energy density. The authors showed that the handheld homogenizer did not significantly downsize the PSD of MPC90 and MC. Only HPH shifted the PSD towards casein micelle size, although, the particle sizes were still bimodally-dis-

tributed. However, the lowest necessary shear impact for full MPC90 and MC dissolution has not been identified. Since a handheld homogenizer does not run continuously, it is hardly transferable to industrial shear systems.

In the dairy industry, two high shear units are widely implemented and can be used for powder redispersion: shear pumps like in powder mixers and HPH. The shear conditions, such as shear rate, turbulent flow, and cavitation (only in HPH) are involved in particle destruction. Therefore, we performed experiments on a laboratory scale first, using a colloid mill as rotor/stator system and a laboratory scale HPH. For the subsequent upscaling experiments a shear pump and a pilot scale HPH were used.

To the best of our knowledge, a study dealing with the impact of high shear conditions on the protein functionality MC- and MPC-enriched skim milk in terms of rennet gelation behavior has never been reported so far. We hypothesized that remaining powder particles of poorly soluble MC88 and MPC85 impair the rennet gelation behavior due to inaccessible proteins in the powder aggregates as well as the gel properties, which could be disturbed by large powder particles.

To evaluate the impact of remaining powder aggregates on the protein functionality, the rennet gelation behavior of the MC88- and MPC85-enriched skim milk was investigated. MC88 and MPC85 differ in protein composition and presence of low molecular solutes, which are known to have an impact on speed and completeness of milk powder rehydration. For the experiments, skim milk was enriched with either MC88 or MPC85 to a casein concentration of 4.5% (w/w), which is in the range typical for cheesemaking (Harvey, 2006). The assessment criteria were particle size as a function of shear rate and the rennet gelation properties. As for cheese manufacture, gelling time, gel strength, structure loss upon deformation, and serum loss were measured. Furthermore, the casein, whey protein, and casein macropeptide (CMP) recovery in the sweet whey was determined to evaluate the shear- and hence, particle size-dependent protein accessibility.

This study should provide insights into the effect of incomplete rehydration of added milk protein powders of various composition on the rennet gelation behavior of powder-enriched skim milk. This enables producing cheese from protein-enriched milk without filtration, but with the same characteristics as retentates. Depending on the powder type, the composition can be varied, and the functionality of the protein-enriched milk can be enhanced. With upscaling experiments, we can recommend suitable shear conditions for best powder dissolution and protein functionality on laboratory as well as on pilot scale.

3.2.2. Material and Methods

3.2.2.1. MC88- and MPC85-enriched skim milk

Pasteurized skim milk (74 °C, 28 s) from the local dairy (Molkerei Weihenstephan GmbH & Co. KG, Freising, Germany) was either enriched with micellar casein concentrate powder containing 88% (w/w) total protein (MC88) or with milk protein concentrate powder containing 85% total protein (MPC85) purchased from MILEI GmbH, Leutkirch im Allgäu (commercial name TMP85). Both powders were used before the best before date. The casein concentration was adjusted to 4.5% (w/w), which is in the range typical for cheesemaking (Harvey, 2006). The powder compositions are presented in Table 3.3.

Table 3.3. Powder compositions of MC88 and MPC85 (mean ± standard deviation).

	Casein (%, w/w)	Whey protein (%, w/w)	Total protein (%, w/w)	Casein/ whey protein ratio	Degree of whey protein denaturation (%)	Lactose (%, w/w)	Minerals (%, w/w)	Total solids (%)
MC88	78.4 ± 0.5	5.6 ± 0.6	84	93:7	65.4 ± 0.8	1.4 ± 0.2	2.7 ± 0.0	94.4 ± 0.0
MPC85	70.5 ± 0.2	11.5 ± 0.1	82	86:14	42.7 ± 0.0	2.7 ± 0.0	2.4 ± 0.0	94.6 ± 0.0

Casein and whey protein content (according to Dumpler et al., 2017) and lactose content (according to Schmitz-Schug et al., 2013) were determined by reversed-phase high performance liquid chromatography (RP-HPLC). The protein concentrations given in Table 3.3 represent the pure protein fractions without peptides; hence, the total protein content, which consists of both, protein fractions and peptides, is lower than 88 and 85%. The flame photometer ELEX 6361 (Eppendorf AG, Hamburg, Germany) was used to measure the amount of soluble minerals (Na^+, K^+) and the total Ca^{2+} concentration in the rehydrated powders. All powders were stored in aluminum compound foil bags at 20 °C to avoid oxygen migration through the packaging material and to prevent the powder from UV radiation.

3.2.2.2. Shear treatments

The impact of shear in a rotor/stator system as well as in a high-pressure homogenizer (HPH) on the particle size was investigated. For shear treatments on a laboratory scale a colloid mill (IKA Laboratory Pilot 2000/4, IKA-Werke, Staufen im Breisgau, Germany) equipped with a radial impeller MK module and a laboratory HPH (APV 1000, SPX Flow Technology, Crawley West Sussex, United Kingdom) were used. The corresponding systems in the upscaling experiments were the shear pump FSP 712/124 (FRISTAM Pumpen KG (GmbH & Co., Hamburg, Germany) and the pilot HPH Rannie 56 type 16.56H with a single stage valve (APV Gaulin GmbH, Lübeck, Germany). In all cases, the samples were sheared in single path, imitating an industrial, continuous process.

Before the shear treatments, one batch each of MC88- and MPC85-enriched skim milk, respectively, was produced. For this, the powders were pre-dissolved in the milk for 30 min at 40 °C under steady stirring at 27 s^{-1}. At pilot scale level, we produced 200 kg pre-mixture in a tempered double-walled cream maturing tank (d_T = 80 cm, Inox Behälter GmbH, Delmenhorst, Germany) equipped with an anchor stirrer (d_i = 76 cm, W = 6.4 cm). On laboratory scale, we produced 1300 g using a metal tank (d_T = 16 cm) placed in a water bath and stirred with an overhead stirrer (IKA MINISTAR 80 digital, IKA-Werke, Staufen im Breisgau, Germany) equipped with an anchor stirrer as well (d_i = 15 cm, W = 2 cm). The shear rate $\dot{\gamma}$ (s^{-1}) in a stirred tank was calculated by Equation (3.2) according Bowen (1986):

$$\dot{\gamma} = 4.2 \cdot N \cdot \left(\frac{d_i}{d_T}\right)^{0.3} \cdot \frac{d_i}{W} \quad (3.2)$$

Where N was the stirrer speed (s^{-1}), d_i was the diameter of the stirrer (m), d_T the diameter of the tank (m), W the width of the stirrer blade (m), and 4.2 and 0.3 were empirically determined factors.

The samples were treated at four different shear rates in the colloid mill/shear pump which was determined according to Equations (3.3) and (3.4).

$$\dot{\gamma} = \frac{dv}{dh} = \frac{2v}{h} \tag{3.3}$$

where v is the flow velocity (m s^{-1}) and h the gap width (m). v is defined as

$$v = 2\pi rf \tag{3.4}$$

where r is the radius of the stator (m) and f the frequency of the rotor (s^{-1}).

The shear rate, which induces the aggregates' destruction in the high-pressure homogenizer gap can be calculated by Equation (3.5):

$$\dot{\gamma} = \frac{2\,\dot{V}}{\pi\, d_{eff}\, h^2} \tag{3.5}$$

$\dot{V}$ is the flow rate (m^3 s^{-1}), d_{eff} the efficient diameter (m) and h the gap height (m).

The configurations for the desired shear rate of the stirred tanks, colloid mill, shear pump, and high-pressure homogenizers are presented in Table 3.4.

Table 3.4. Configurations chosen for shear treatments in laboratory and pilot scale for the same shear rate calculated with Equations (3.2)-(3.5).

	Stirred tank	Colloid mill/ shear pump	Colloid mill/ shear pump	Colloid mill/ shear pump	Colloid mill/ shear pump	High-pressure homogenizer
Configuration laboratory scale	53 rpm	3170 min^{-1}	3487 min^{-1}	4026 min^{-1}	5088 min^{-1}	100 bar
Configuration pilot scale	33 rpm	1255 min^{-1}	1381 min^{-1}	1594 min^{-1}	2015 min^{-1}	100 bar
Calculated shear rate (s^{-1})	27	$3.3x10^3$	$3.6x10^4$	$4.1x10^4$	$5.2x10^4$	$6.0x10^7$

3170 min^{-1} was the lowest possible rotation speed for the colloid mill; hence, the minimum possible shear rate was $3.3x10^3$ s^{-1}. In preliminary experiments, it turned out that after treating the samples at shear rates higher than $5.2x10^4$ s^{-1} and $6.0x10^7$ s^{-1}, respectively, the particle sizes did not change with increasing shear rate (Figure 3.7). Therefore, the maximum shear rate chosen for the rotor/stator system was $5.2x10^4$ s^{-1} and for the high-pressure homogenizer, it was $6.0x10^7$ s^{-1} (corresponding to 100 bar). Each experiment was performed in duplicate.

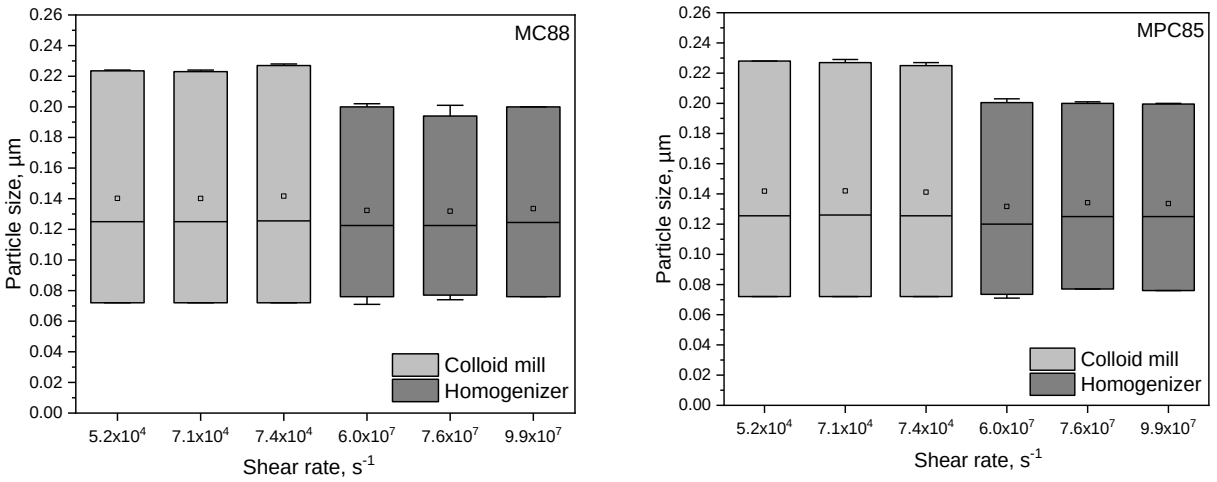


Figure 3.7. d_{10}, d_{50} and d_{90} represented as boxplots of skim milk enriched with MC88 (a) and MPC85 (b) after homogenization with colloid mill and high-pressure homogenizer at shear rates ≥ $5.2x10^4$ and ≥ $6.0x10^7$ s^{-1}, respectively. The low and high quartiles, the median, and the mean (□) are plotted.

3.2.2.3. Particle size measurements

Particle sizes were measured by static light scattering using a Malvern Mastersizer 2000 equipped with a Malvern Hydro 2000S sample dispersion unit (Malvern Instruments GmbH, Herrenberg, Germany). Particle sizes are calculated within 0.02 - 2000 µm of up to 100 size classes applying Mie theory (Rawle, 2003; Rawle and Kippax, 2010). This allows measuring on the one hand casein micelles and on the other hand large powder particles at once. This method has been used by many studies for similar purposes (Anema et al., 2014; Bouvier et al., 2013; Chandrapala et al., 2014; Crowley et al., 2015; Ferrer et al., 2008; Ji et al., 2016; Kieferle et al., 2019; Sandra and Corredig, 2013; Warncke and Kulozik, 2020). Hence, it is established for measuring milk powder solubility.

The refractive indices of the dispersant (deionized water) and the protein were 1.33 and 1.41, respectively. The particle absorption index was 0.001 (according to Dumpler and Kulozik, 2016). The sample was added and dispersed at a constant stirrer speed (2000 rpm) until the obscuration reached 15 ± 1% according to the guidelines of the manufacturer. The stirring prevents the large powder particles from sedimentation. Besides the distribution density $q_3(x)$ and the cumulative distribution $Q_3(x)$, the software calculates the related $d_{10,3}$, $d_{50,3}$, and $d_{90,3}$ values, meaning 10, 50 and 90% of the particles are smaller than the respective d-value. Fresh skim milk was the reference. Each sample was measured in duplicate at 20 °C within 3 min.

3.2.2.4. Rennet gelation properties

To evaluate the impact of remaining powder aggregates on the protein functionality, the rennet gelation behavior of the MC88- and MPC85-enriched skim milk was investigated. For this, the MCR 702 rheometer (Anton Paar GmbH, Graz, Austria) equipped with the concentric cylinder geometry CC27 was used. 14.7 mL of the unrenneted sample was tempered to 40 °C and simultaneously sheared at 100 s^{-1} for 5 min to give the sample time to equilibrate. After reaching and holding 40 ± 0.02 °C for 1 min, the rennet (CHY-MAX® M 1000, Chr. Hansen A/S, Hørsholm, Denmark) with an enzyme activity of 1000 IMCU L^{-1} (international milk clotting

units) was added with a concentration of 2.303 µL per gram casein. The rennet was mixed in immediately by shearing the sample at 500 s^{-1} for 10 s before resting for 3 s. Oscillation at a constant deformation (0.01 %) and frequency (1 Hz) was applied for 30 min, whereby the sample formed a gel. The onset of gelation was defined as the point where the storage modulus G′ exceeded 1 Pa. The gelation time was defined as the time until the onset of gelation occurred.

33 min after rennet addition, the oscillation followed an amplitude sweep (logarithmic ramp from 0.01 to 100% deformation at a constant frequency of 1 Hz) to determine the gel strength in the linear viscoelastic region (LVR) (corresponds to 0.01-0.1% deformation within 2.5 min) and the structure loss upon deformation. The gel strength corresponded to the mean value of the measured storage modulus G′ (Pa) between 0.01-0.1% deformation. G′ is defined as the elastic portion of a sample which increases with increasing gel strength. Therefore, it can be directly related to the gel strength obtained by oscillatory measurements. The structure loss is of interest for the curd cutting, which should be smooth-running. The structure loss was calculated as follows (Equation (3.6)):

$$Structure\ loss\ (\%) = \frac{G'_{100\%}}{G'_{0.01\%}} \cdot 100\% \tag{3.6}$$

where $G'_{0.01\%}$ is the storage modulus at 0.01% deformation (Pa) and $G'_{100\%}$ is the storage modulus at 100% deformation (Pa). All samples were measured in duplicate.

3.2.2.5. Serum loss and casein, whey protein, and casein macropeptide recovery in the sweet whey

The whey drainage or serum loss, which occurs upon curd cutting and pressing, is an important criterion in cheese manufacture which defines the dry matter and therefore, the hardness of the final cheese. For serum loss determination, 30 ± 0.9 g of the unrenneted samples were weighed in duplicate into 50 mL centrifuge tubes and placed in a 40 °C-tempered water bath. After reaching 40 ± 0.1 °C, rennet was added in the same concentration as used for the rheological measurements. After 1 h of incubation, the samples were centrifuged at 4000 *x* g for 45 min at 20 °C. The supernatant was immediately weighed, and the serum loss calculated by Equation (3.7), where m_{serum} (g) is the serum mass and m_0 (g) the mass of the whole sample before renneting.

$$Serum\ loss\ (\%) = \frac{m_{serum}}{m_0} \cdot 100\% \tag{3.7}$$

To evaluate whether the protein accessibility is impaired due to remaining nor fully rehydrated powder aggregates, the casein, whey protein, and casein macropeptide (CMP) concentrations in the sweet whey were analyzed by RP-HPLC. The protein recovery (%) indicates how much of each protein fraction found in the MC88 and MPC85-enriched skim milk is found in their supernatants after renneting and centrifuging. Since unrenneted skim milk does not contain CMP, the CMP concentrations of the renneted high-pressure-homogenized and fully solubilized MC88- and MPC85-enriched skim milks were set as relative values for CMP recovery calculation. We expected that more complete powder solubilization results in a better protein accessibility. Hence, the concentrations of whey protein and CMP in the serum phase should be higher.

3.2.2.6. Statistical analyses

Origin 2020 (OriginLab Corporation, Northampton, United States) was used to plot graphs and RStudio, Inc., 2019 (version 1.2.5033, Boston, MA, United States) was used for statistical analysis. Statistical significances were evaluated using one-way analysis of variance (ANOVA) combined with Tukey's HSD post-hoc test. The calculated *P*-values and the respective significance levels are given in the text ($P \leq 0.001$, $P \leq 0.01$, $P \leq 0.05$, $P \leq 0.1$).

3.2.3. Results and discussion

3.2.3.1. Particle size distributions of protein-enriched skim milk as a function of shear rate

To evaluate the impact of high shear applied with colloid mill ($3.3x10^3$-$5.2x10^4$ s^{-1}) and high-pressure homogenizer (HPH) ($6.0x10^7$ s^{-1}) on the powder solubility, we measured the particle sizes after the shear treatments by static light scattering.

Figure 3.8 presents the cumulative particle size distributions (PSD) and the $d_{10,3}$, $d_{50,3,}$ and $d_{90,3}$ values illustrated as boxplots for skim milk enriched with MC88 (a,b) and MPC85 (c,d) in comparison to skim milk without powder addition as reference. The target was to achieve the same monomodal PSD as the reference, which represents the PSD of the natural casein micelles. The particle size distribution of the fresh skim milk was in accordance with Dumpler et al. (2017) and Sandra and Corredig (2013) (Figure 3.8 a,c). The corresponding $d_{10,3}$, $d_{50,3}$, and $d_{90,3}$ values were 70, 124, and 212 nm, respectively (Figure 3.8 b,d). The volume-weighted mean diameter $d_{4,3}$ was 133 nm, which was also reported by Ferrer et al. (2008) and Warncke and Kulozik (2020). As expected, the particle sizes in MC88- as well as in MPC85-enriched skim milk decreased with increasing shear rate. $3.3x10^3$ and $3.6x10^4$ min^{-1} showed the worst dissolution results as their mean particle sizes were around 30 µm and their $d_{50,3}$ varied between 0.4 and 0.2 µm (Figure 3.8 b,d). A shear rate of at least $4.1x10^4$ s^{-1} markedly shifted the PSD towards smaller particle sizes. The mean particle size in both samples conformed to casein micelles (~0.15-0.2 µm). However, even the highest shear in the colloid mill ($5.2x10^4$ s^{-1}) was insufficient to fully dissolve the powders as the distributions were still bimodal (Figure 3.8 a,c). As already shown in section 3.2.2.2., a further shear rate increase did not improve the powder dissolution. Only HPH was able to destruct all remaining agglomerated powder particles and to achieve a monomodal particle size distribution as found for skim milk with a corresponding $d_{50,3}$ of 0.124 µm. This leads to the assumption that laminar or turbulent flow alone, even at high shear rates, is insufficient to completely disintegrate MC88 and MPC85 powder agglomerates and that cavitation, as it occurs in HPH, is a decisive factor for solubilizing high protein powders.

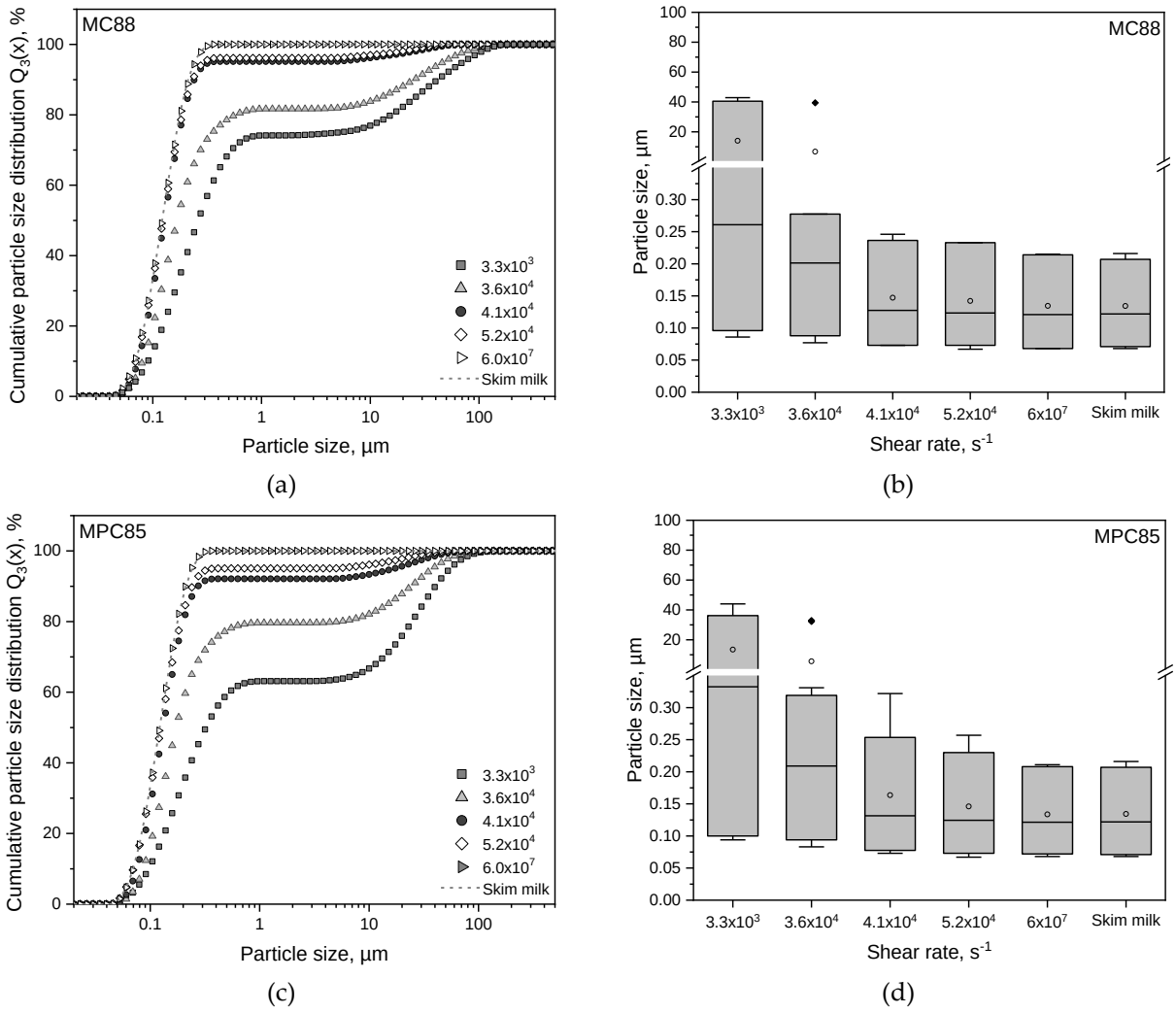


Figure 3.8. Cumulative particle size distributions $Q_3(x)$ (volume % vs. diameter) (left) and $d_{10,3}$, $d_{50,3}$, and $d_{90,3}$ presented as boxplots (right) of skim milk and skim milk enriched with MC88 (a,b) and MPC85 (c,d) after homogenization with colloid mill ($3.3x10^6$ – $5.2x10^4$ s^{-1}) and high-pressure homogenizer ($6.0x10^7$ s^{-1}) at 40 °C. Boxplots: The low and high quartiles, the median, and the mean (□) are plotted. (◆) represents outliers.

MC88 was slightly better soluble than MPC85. It is said that caseins are the so-called slow dissolving components in milk protein powders, whereas whey proteins and lactose are considered as fast dissolving components. Warncke and Kulozik (2020) identified lactose as the deciding fast dissolving component regarding powder solubility. It is important to note that the better soluble MC powder used in that study contained as much casein as the used MPC85, but 13.4% lactose compared to 4% in the MPC85. In the present study, the lactose concentrations of both powders were low; 1.4 and 2.7%, respectively. Although MC88 contained less fast dissolving components than MPC85, it showed a better rehydration behavior in the shear range of $3.3x10^3$ to $4.1x10^4$ s^{-1}. Since the protein as well as the lactose concentrations in both powders were similar, our findings indicate that the casein/whey protein ratio, which in turn defines the extent of protein interactions and aggregate formation, determines the powders' dissolution behavior.

Protein interactions and whey protein denaturation occurring during powder processing may be involved. According to Oldfield and Singh (2005), the extent of protein interactions that occur upon pre-heating

before evaporation and spray drying in milk powder production affects the powder solubility and shelf life. The higher the degree of whey protein denaturation and aggregation, the better the oxidative stability of whole milk powders and the worse their solubility. Oldfield et al. (2005) could show that evaporating skim milk up to 49% total solids and heat treating the concentrate at 64-74 °C did not significantly affect the whey protein denaturation. The authors explained this by the increased stability of the whey proteins at high total solid contents. We postulate that this could also be related to the high lactose concentration instead of the total solid content, and therefore, to the constantly high lactose/protein ratio during evaporation. In the presence of sugars, whey proteins prefer the associated form to avoid unfavorable water-protein interactions (Arakawa and Timasheff, 1982; Timasheff, 2002). Thus, lactose has a protective effect on β-lactoglobulin against denaturation if the lactose/protein ratio is high enough (Bernal and Jelen, 1985; Garrett et al., 1988; Jou and Harper, 1996; Plock et al., 1998; Spiegel, 1999). However, milk protein concentrates for high protein powder production are not pre-heated, but usually evaporated and spray-dried directly after diafiltration (Singh, 2007). In the case of diafiltered milk protein concentrates the lactose concentration is much lower. Warncke et al. (2022) observed no differences in the degree of whey protein denaturation of 0, 37, and 88% whey protein-depleted milk protein concentrates (diafiltered with ultrafiltration permeate) at the same total protein concentration after heating for 30 min at 80 °C. This was explained by the unaltered amount of reactive binding sites for the whey proteins (other whey proteins as well as the surfaces of casein micelles) in all three samples at the same total protein concentration.

As a result, the collision probability of the whey proteins with either casein micelles or other whey proteins was similar. Thus, differences in the degree of whey protein denaturation cannot be attributed to the casein/whey protein ratio alone. Warncke et al. (2022) observed as well that a high whey protein ratio went hand in hand with an extensive whey protein aggregate growth in the serum phase. The strong disulfide bonds between the whey proteins and between the whey proteins and the κ-casein seem to be responsible for the poorer solubility of MPC85 compared to MC88 although the whey protein denaturation degree in MPC85 was 42.7 ± 0.0% and in MC88 65.4 ± 0.8%. Since this is a ratio, it is not indicating the number of formed aggregates and is therefore not a suitable measure to evaluate the powder solubility. Considering the casein/whey protein ratio as a factor could be more revealing:

The more whey proteins a powder contains, the more heat-induced aggregates form upon heating (in the evaporator and spray dryer), and the worse is the powder solubility. McKenna (2000) and Havea (2006) identified the insoluble material in MPC85 predominantly as fused α_s- and β-caseins, forming a skin-like structure on the powder particles' surface, which inhibits the water penetration. Disulfide-linked κ-casein/β-lactoglobulin complexes were present as well but not considered to play an important role in the formation of insoluble material. Our results presented in Figure 3.8 indicate that whey protein aggregates as well as casein/whey protein complexes, which are more present in MPC85 than in MC88, are less soluble than casein aggregates, and are, hence, the least dissolving components. Therefore, MPC85 required higher shear forces for aggregates' destruction and fully powder solubilization than MC88.

3.2.3.2. Functionality of MC88- and MPC85-enriched skim milk: rennet gelation behavior

In the following chapter, the rennet gelation behavior of MC88- and MPC85-enriched skim milk is presented.

3.2.3.2.1. Gelation time

The gelation time is defined as that time, which the gelling system requires to reach a storage modulus G′ of 1 Pa (≙ onset of gelation). Figure 3.9 illustrates the gelation time as a function of the mean particle size $d_{4,3}$.

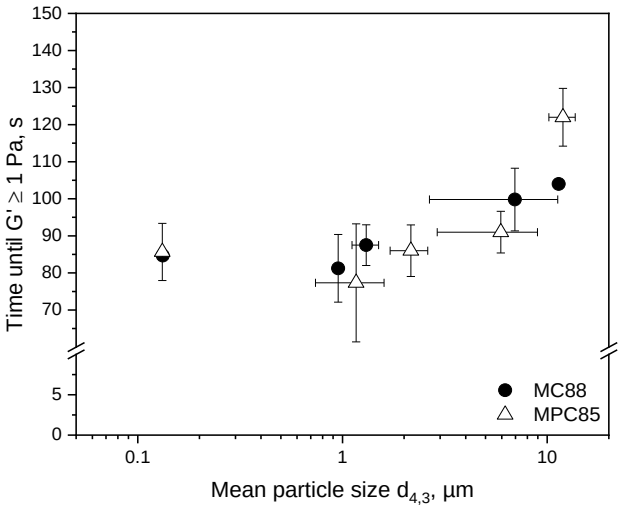


Figure 3.9. Time until the onset of gelation (G′ ≥ 1 Pa) of skim milk enriched with MC88 and MPC85 as a function of mean particle size $d_{4,3}$.

As shown before, the particle size decreased with increasing shear rate (Figure 3.8). Therefore, the largest particles correspond to the lowest shear rate and the smallest particles to the highest shear rate. Figure 3.9 shows that the gelation time decreased with decreasing particle size. MPC85 required ~120 s to reach the onset of gelation when sheared at 3.3×10^3 s^{-1} (mean particle size around 10 µm) and MC88 needed ~115 s at the same shear rate and particle size. On the contrary, HPH led to a mean particle size of 0.13 µm in both powder-enriched milks and reduced the gelation time to 85 s. This is in accordance with Martin et al. (2008), who observed a faster rennet gelation of reconstituted skim milks increasing in rehydration time. Although the particle sizes were not measured, it can be assumed that the particle size was also decreasing with increasing rehydration time. These results prove that insufficient powder rehydration prolongs the rennet gelation time, with the result that the curd may be cut too early in the renneting process. This may lead to fluctuations in the resulting cheese properties and quality.

The impact of whey protein denaturation on the gelation time is negligible when adding MPC85 to skim milk in that concentration used in this study since MPC85-enriched skim milk did not show significantly longer gelation times ($P \leq 0.1$) than MC88-enriched milk.

3.2.3.2.2. Gel strength and casein, whey protein, and CMP recovery in the sweet whey

The gel strength in the linear viscoelastic region (LVR) was determined by rheometry. This method provides insights regarding gel firmness without disturbing the gel network like in penetration measurements.

The gel strength displays the curd firmness after full formation before cutting. We observed that the gel strength in the LVR increased with decreasing particle size and hence, increasing shear rate (Figure 3.10).

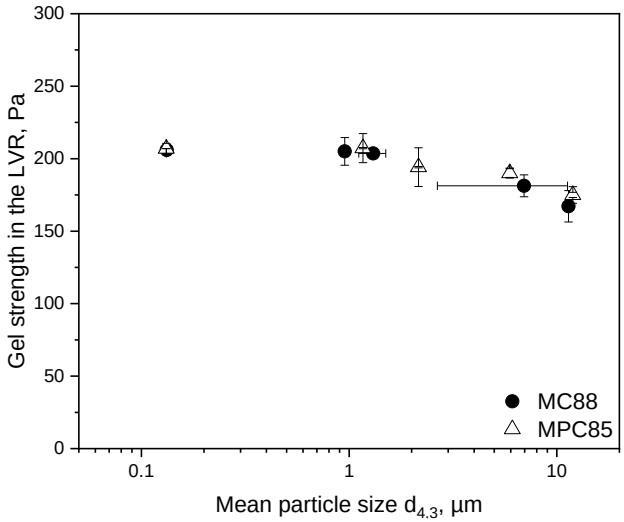


Figure 3.10. Gel strength in the linear viscoelastic region (LVR) of skim milk enriched with MC88 and MPC85 as a function of mean particle size $d_{4,3}$. Particle sizes decrease with increasing shear rate.

Our results indicate that the gel strength is particle size-dependent since we could show that the gelling time and gel strength of skim milk enriched with powders, whose whey protein denaturation degrees were around 50%, can be enhanced by higher shear treatments and particle size reduction. Hence, the insufficient rehydration of MPC90 in the study of Ferrer et al. (2008) and therefore, the impaired casein micelles' accessibility for the rennet may be responsible for the lower gel strength rather than the whey protein denaturation itself. The lower CMP release of MPC90 compared to MPC56 and MPC70 observed by Ferrer et al. (2008) supports our suggestion.

We further investigated the protein concentration (casein, whey protein, and CMP) in the whey after renneting as well to evaluate the protein accessibility as a function of shear rate and particle size. On the one hand, smaller particle sizes should improve the transition of the whey proteins into the sweet whey; on the other hand, the CMP concentration should increase as well due to the separation of the casein micelles from the casein micelle agglomerates in the powder particles.

Figure 3.11 illustrates the casein, whey protein, and CMP recovery in the sweet whey as a function of particle size.

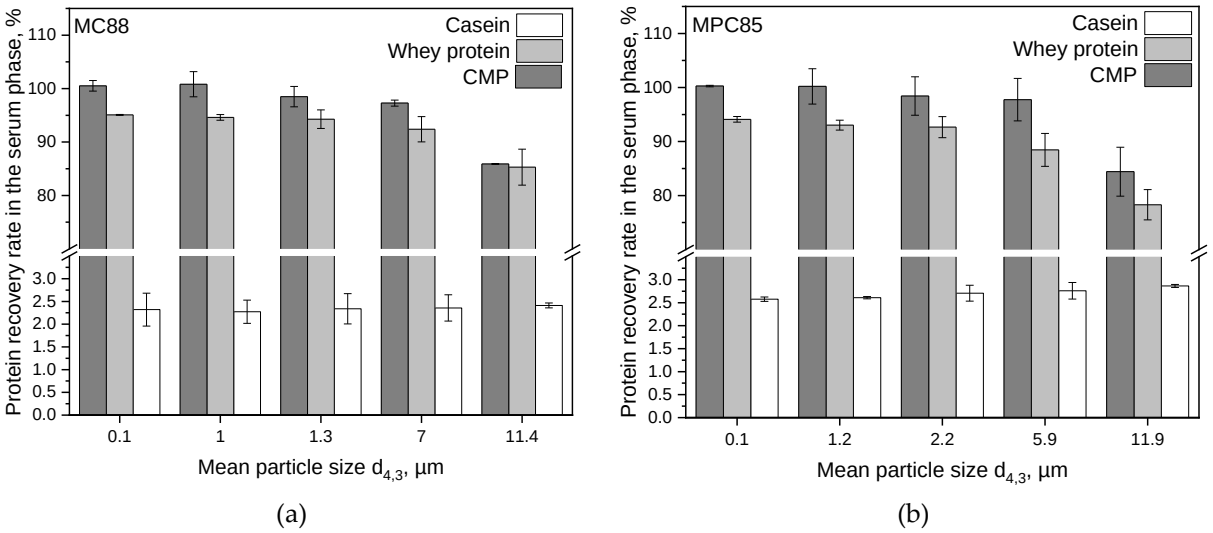


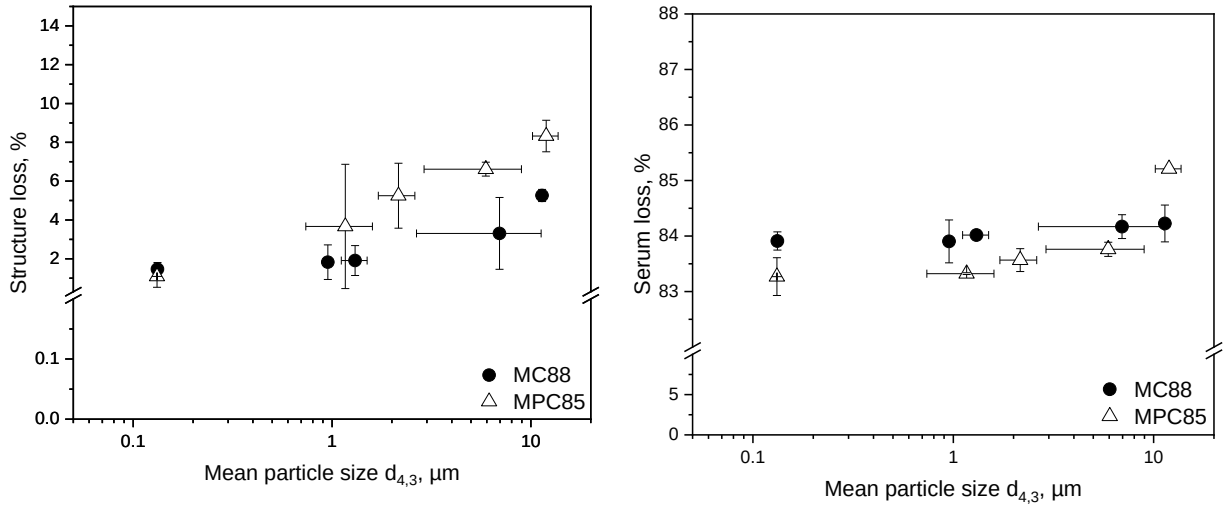
Figure 3.11. Casein, whey protein, and casein macropeptide (CMP) recovery in the serum phase after incubation (t = 1 h, ϑ = 40 °C) and centrifugation at 4000 *x* g (t = 45 min, ϑ = 20 °C) of skim milk enriched with MC88 (a) and MPC85 (b) plotted for each mean particle size $d_{4,3}$.

In both samples, we observed an increased whey protein and CMP recovery in the whey with decreasing particle size, which meets our expectations. Increasing the shear rate from 3.3×10^3 s^{-1} to 3.6×10^4 s^{-1} resulted in smaller particles but also in a significantly higher ($P \leq 0.01$) whey protein and CMP recovery in the sweet whey. The casein recovery did not significantly change with increasing shear rate ($P \leq 0.1$) and was constantly around 2.5% in both samples. The CMP recovery is a measure for the degree of hydrolysis. Since the CMP concentrations of the samples sheared at 6.0×10^7 s^{-1} were set as relative, maximum possible values, the CMP recovery was 100% in both samples at that shear rate. It turned out that none of the samples treated at lower shear rates achieved 100% CMP recovery. This implies that a notable, particle size-dependent amount of κ-casein was not hydrolyzed, and the related CMP was consequently not found in the supernatant resulting in its lower recovery. These findings prove that the protein recovery depends on the rehydration level of the added powders only. Or, in other words, on the powder particle destruction and consequently on the better casein micelles' accessibility for the rennet. Native whey proteins are not involved in the rennet gelation process, but most of them drain with the sweet whey. A small amount remains in the pores of the network. Their recovery in the sweet whey is also a measure for evaluating the powder solubility. The more whey proteins remain bound in powder particles, the more are involved in the gel network and the less are freely soluble in the whey and drain with the serum phase.

3.2.3.2.3. Structure loss upon deformation and serum loss

After curd formation within a given set-to-cut time, the curd is cut to induce serum loss/syneresis. The serum loss defines the cheese moisture and depends on the cube size, cooking temperature, and applied pressure. It is important that the curd has the optimal firmness so it can withstand the mechanical action of the cutting knives without shattering (Fox et al., 2017). A too soft or rigid texture causes shattering and curd fines formation (particles < 1 mm) which get lost with the whey (Fox et al., 2017). Consequently, the cheese yield decreases.

The structure loss is a measure for the fragility of the rennet gel. The structure loss upon deformation determined by rheometry is presented in Figure 3.12 a.



(a) (b)

Figure 3.12. Structure loss during amplitude sweep ranging from 0.01 to 100% deformation (a) and serum loss after incubation (t = 1 h, ϑ = 40 °C) and centrifugation at 4000 *x* g (t = 45 min, ϑ = 20 °C) (b) of skim milk enriched with MC88 and MPC85 as a function of mean particle size $d_{4,3}$. Particle sizes decrease with increasing shear rate.

In both samples, the structure loss decreased with increasing shear rate and decreasing particle size. If the powders are not completely dissolved (particle sizes > 0.124 µm), the MPC85-enriched skim milk show higher structure losses than the MC88 samples. The structure loss allows conclusions to be drawn about the gel strength or deformation resistance outside the LVR. The results indicate that MC88-enriched skim milk has a higher deformation resistance than MPC85-enriched skim milk. The reason is the slightly higher calcium concentration of 2.4% in MC88 compared to 2.1% (w/w) in MPC85. The dissolution medium's composition (fresh skim milk in this case) was constant for both powders. Calcium is involved in the gel formation by forming calcium bridges between the renneted casein micelles, which in turn, defines the final curd firmness (Lucey and Fox, 1993; Udabage et al., 2001). A low calcium concentration in the milk goes hand in hand with less calcium bridges and consequently, with a higher structure loss upon deformation. However, after HPH the structure loss of the MC88 and MPC85 samples aligned (~1.5% in both samples) at a particle size of ~0.1 µm. These results clearly demonstrate that renneted MC88- as well as MPC85-enriched skim milk become more resistant against deformation with decreasing particle size, whereas larger particle sizes result in a more fragile gel network. It could be expected that the brittle texture of the MPC85 samples homogenized with the colloid mill results in a higher serum loss because this is favored by a large surface area.

Figure 3.12 b illustrates the serum loss of MC88- and MPC85-enriched skim milk gels after centrifugation. The serum loss of both samples decreased with decreasing particle size. As expected, MPC85-enriched skim milk showed at a $d_{4,3}$ of ~10 µm the highest serum loss with 85.2%. Between 0.1 and 8 µm the serum loss varied between 84 and 83%. The differences between MC88 and MPC85 were insignificant ($P \leq 0.1$).

3.2.3.3. Transferability from laboratory to pilot scale: Correlation between shear rate and particle size

In order to demonstrate that the results found on a laboratory scale are transferable to pilot scale, we performed upscaling experiments. We wanted to assess whether the particle sizes obtained on a laboratory scale are obtained on a pilot scale as well when homogenizing the samples at the same shear rates. For this, a shear pump as rotor/stator system and a pilot HPH was used. However, it turned out that the particle sizes in MC88- and MPC85-enriched skim milk treated in the shear pump are higher compared to the particle sizes after colloid mill treatment in laboratory scale (Figure 3.13 a,b). Above $4.1x10^4$ s^{-1} the $d_{10,3}$, $d_{50,3}$, and $d_{90,3}$ values of MC88-enriched skim milk homogenized on a laboratory and a pilot scale were similar (Figure 3.13 a). On the contrary, even the harshest conditions in the shear pump were insufficient to completely dissolve MPC85 (Figure 3.13 b). Only HPH at 100 bar led to the same particle sizes as found on a laboratory scale.

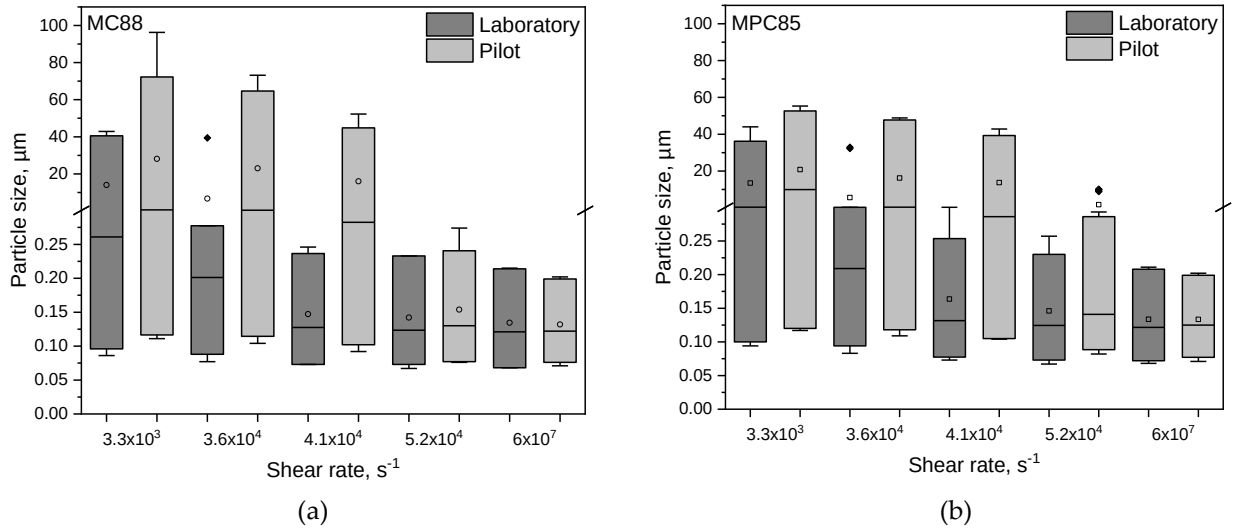
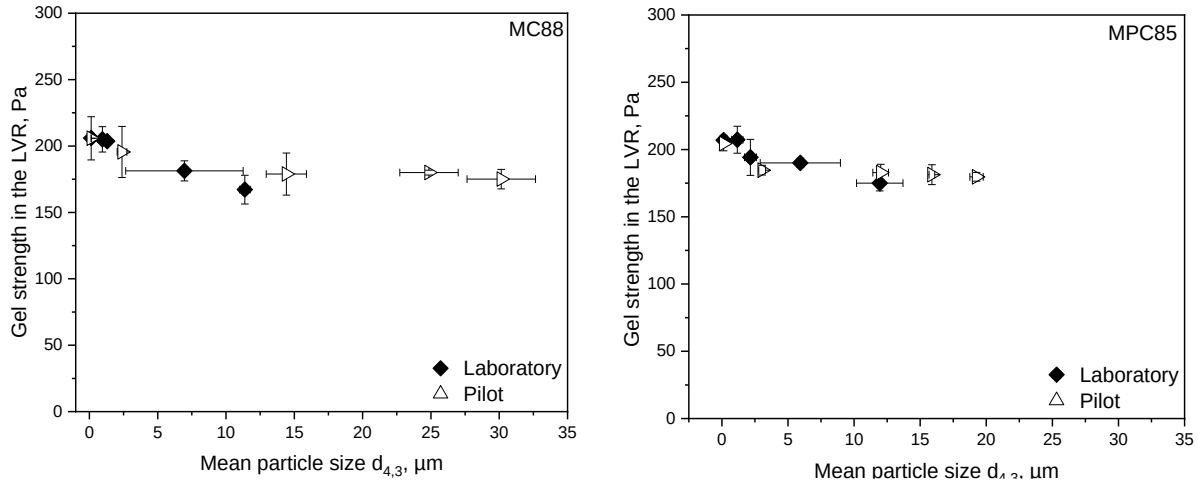


Figure 3.13. $d_{10,3}$, $d_{50,3}$, and $d_{90,3}$ values observed in laboratory (dark grey) and pilot scale (light gray) presented as boxplots of skim milk enriched with MC88 (a) and MPC85 (b). The low and high quartiles, the median, and the mean (□) are plotted. (◆) represents outliers.

The larger particle sizes indicate that the powder particles undergo less shear in the pump than in the laboratory colloid mill although the shear rates were calculated in the same way. Nevertheless, the rennet gelation behavior was in accordance with the laboratory results. Figure 3.14 shows exemplarily for the gelling behavior the gel strength in the LVR of MC88- and MPC85-enriched skim milk sheared in laboratory and pilot scale.



(a) (b)

Figure 3.14. Gel strength in the linear viscoelastic region (LVR) of skim milk enriched with MC88 (a) and MPC85 (b) produced in laboratory and pilot scale as a function of mean particle size $d_{4,3}$. Particle sizes decrease with increasing shear rate.

In both cases, the gel strength decreased further with increasing particle sizes. Due to the clear particle size-dependent trends—independent from laboratory or pilot scale—we wanted to calculate the "true" shear rates appearing in the shear pump based on the laboratory results. The "true" shear rates (meaning the shear rates which actually occurred) in the shear pump seem to be lower than in the colloid mill, although the applied shear rates were calculated in the same way. For the calculations, high shear (colloid mill) and low shear (overhead stirrer) was applied on skim enriched with either MC88 or MPC85 to obtain a fitting curve covering a wide shear rate range. Equation (3.8) presents the fit equation and Table 3.5 the corresponding fit parameters. The curve is illustrated in Figure 3.15.

$$y = y_0 + a \cdot e^{(b \cdot x)} \tag{3.8}$$

Table 3.5. Fit parameters y_0, a, and b of modeling the shear rate in laboratory scale by the fit equation.

y_0	a	b
7.32755	8.034×10^4	-0.27206

Based on the particle sizes, the "true" shear rates of the pilot results could be calculated by Equation (3.8). Doing so, the pilot results (black circles) correlated perfectly with the laboratory fit (Figure 3.15).

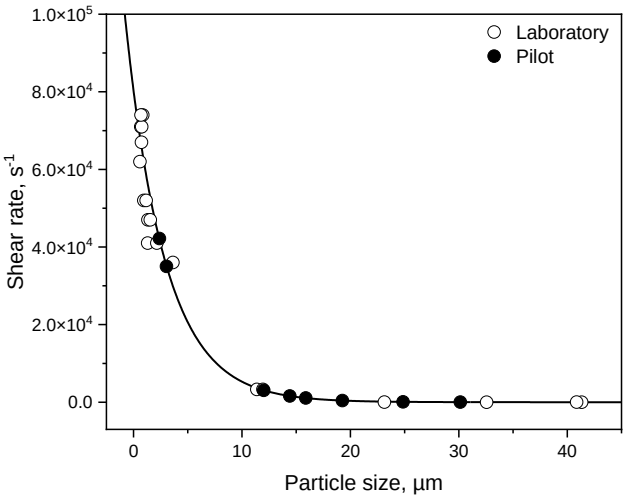


Figure 3.15. Correlation between calculated shear rates and observed particle size in laboratory scale of MC88 and MPC85 (white circles) homogenized via colloid mill. Black circles represent the particle sizes observed in pilot scale after homogenization with shear pump. Corresponding shear rates are calculated with the correlation function. The correlation coefficient R^2 of the fit was ≥ 0.99.

The following Table 3.6 shows the corresponding calculated shear rates compared to the targeted shear rates calculated by Equations (3.3)-(3.4). At $3.6x10^4$ s^{-1} the deviation between the shear rates was lowest with 1.6%. In contrast to that, the deviation at $5.2x10^4$ s^{-1} was highest with 74.2%. This means that at this shear rate, the true shear rate was 74.2% lower than desired. Consequently, the particle sizes were larger due to the lower shear intensity than in laboratory scale. In other words, to induce particle sizes of ~1 µm the conditions at pilot scale must be adapted such that the resulting shear rate is 74.4% higher than calculated by Equations (3.3)-(3.4). Thus, the shear rate should be adjusted to $3.9x10^6$ instead of $5.2x10^4$ s^{-1}.

Table 3.6. Shear rates in the shear pump calculated with fit equation (3.8) (mean of MC88 and MPC85 ± standard deviation) compared to targeted shear rates calculated by Equations (3.3)-(3.4). The shear rate deviation between calculated and targeted (percentage of the targeted shear rate) specifies by which shear rate the shear rate calculated by Equations (3.3)-(3.4) had to be increased to reach the targeted shear rates of the pilot scale conditions. This gives the adjusted shear rate settings.

Shear rate calculated by Equation (3.8) (s^{-1})	Targeted shear rate calculated by Equations (3.3)-(3.4) (s^{-1})	Shear rate difference (s^{-1})	Shear rate deviation (%)	Adjusted shear rate settings (s^{-1})
$2.3x10^2 \pm 2.0x10^2$	$3.3x10^3$	$3.1x10^3 \pm 2.0x10^2$	7.0 ± 6.1	$2.3x10^4 \pm 2.9x10^4$
$5.9x10^2 \pm 4.9x10^2$	$3.6x10^4$	$3.5x10^4 \pm 4.9x10^2$	1.6 ± 1.4	$5.9x10^4 \pm 6.9x10^4$
$2.3x10^3 \pm 7.3x10^2$	$4.1x10^4$	$3.9x10^4 \pm 7.3x10^2$	5.7 ± 1.8	$2.3x10^5 \pm 1.0x10^5$
$3.9x10^4 \pm 3.6x10^3$	$5.2x10^4$	$1.3x10^4 \pm 3.6x10^3$	74.2 ± 6.9	$3.9x10^6 \pm 5.1x10^5$

HPH can be taken over from laboratory to pilot scale unrestrictedly; the same pressure induces the same shear rate and hence, the same particle size distribution.

3.2.4. Conclusions

This study demonstrates how the shear rate in laboratory and pilot rotor/stator systems like colloid mill or shear pump and high-pressure homogenizers (HPH) affect the particle sizes and rennet gelation properties of MC88- and MPC85-enriched skim milk.

Applying HPH (100 bar) on poorly soluble dairy powders like MC88 and MPC85 containing high ratios of slow dissolving components appears to be indispensable and sufficient for full powder dissolution. The flow conditions in the rotor/stator systems were insufficient—even at shear rates up to 7.4×10^4 s^{-1}—for complete powder destruction; additional cavitation, which occurs in HPH, was required.

Based on the experimental data, we confirmed our hypothesis that remaining powder particles impair the rennet gelation behavior due to the inaccessible proteins in the powder aggregates as well as the gel properties, which could be disturbed by large powder particles. We could show that insufficient powder rehydration prolongs the rennet gelation time. This could result in too early curd cutting in the cheese making process leading to fluctuations in the resulting cheese properties and quality. Although the gelation time of MC88- and MPC85-enriched skim milk was similar, the addition of MPC85 resulted in softer, weaker gels and consequently, in a reduced syneresis/serum loss. This could be favorable for producing cheeses with high moisture contents. Moreover, remaining powder particles decrease the whey protein concentration in the sweet whey. This can reduce the whey protein yield upon sweet whey purification.

Using HPH is an effective option for powder redispersion, already commonly established in dairies for milk homogenization. Depending on powder characteristics, it could also serve as a method to more effectively redisperse and fully rehydrate more challenging milk speciality powders like MCs or MPCs. Possibly, a toothed disc powder mixer could or would have be installed upstream to HPH in such cases.

Acknowledgments: The authors thank Andreas Matyssek for his assistance in performing the upscaling experiments. Moreover, we wish to express our gratitude to Wolfgang Holzmüller for powder supply and to Claudia Hengst, Heidi Wohlschläger, Hermine Roßgoderer, and Martin Hilz for help in conducting the analytical measurements. Special thanks go to Ingrun Kieferle and Roland Schopf for their support and valuable discussions.

3.3. *Impact of heat treatment, casein/whey protein ratio and protein concentration on rheological properties of milk protein concentrates used for cheese production*

Summary and contribution of the doctoral candidate

In the cheese manufacture it is common to increase the protein content of the vat milk for a higher cheese yield. The protein concentration and composition can be varied via micro- (MF) and ultrafiltration (UF) or by enriching the vat milk with protein powders comprised of milk protein or micellar casein.

The idea of the present study is to produce milk protein powders as base for fresh/hard cheese and quark production already having the optimal composition for manufacturing each of those cheese types. This would require only one rehydration step, which can be performed continuously e.g., with rotor/stator shearing devices or high pressure homogenizers and no further unit operations like protein adjustment or heating; consequently, the production time can be reduced. Another benefit of this process is that the powder manufacturer can remove the whey proteins in advance making the sweet whey collection and purification unnecessary.

In fresh/hard cheese manufacture, a high casein concentration is desired, whereas, for quark production, a high total protein concentration and a high degree of whey protein denaturation is needed. However, concentration impacts inevitably the concentrate's viscosity—particularly after heat treatment. Our research question was therefore, which mechanisms are responsible for changes in viscosity upon increasing the total protein concentration, varying the casein/whey protein ratio, and heating. In order to answer this question, we investigated the viscosity of unheated and heated milk protein concentrates (3-18% total protein) and three casein/whey protein ratios (85:15, 92:8, 98:2).

It turned out that a 37% whey protein depletion was sufficient to 1) inhibit the impact of the casein micelles' repulsive forces on the viscosity; 2) achieve the highest polydispersity and, therefore, particle packing density of all concentrates; and 3) allow up to 95% whey protein denaturation without changing the viscosity of the concentrates after heating. Our results indicate that both heating and concentration are not limiting the viscosity inevitably. A heated concentrate remains fluent and easy to process when whey proteins were partially depleted—even at high total protein concentrations. If a high casein concentration is desired, the maximum total protein concentrating should not exceed 9% to keep the casein micelles' hydration and their repulsive forces on an acceptable level at which the viscosity is still low and unaffected.

The statistical model allows predicting the apparent viscosity of milk protein concentrates of different total protein concentrations and casein/whey protein ratios and how pronounced the differences in viscosity might be after heating. This should help designing processes, such as the protein concentrate powder production used for fresh/hard cheese and quark, where the apparent viscosity is a limiting factor.

The most significant contribution to this manuscript was made by the doctoral candidate. This comprised the conception and design of experiments based on preceded critical literature review as well as major conduction of data analysis, data interpretation, and discussion. In addition, writing and editing of the manuscript was done by the doctoral candidate. The co-authors contributed to the project outline, the execution of experiments, the discussion of results, and the revision of the manuscript.

Adapted original manuscript[5]

Impact of heat treatment, casein/whey protein ratio and protein concentration on rheological properties of milk protein concentrates used for cheese production[6]

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Abstract: In fresh/hard cheese manufacture, a high casein concentration is desired, whereas, for quark production, a high total protein concentration and a high degree of whey protein denaturation is needed. However, concentration impacts inevitably the concentrate's viscosity—particularly after heat treatment. Our research question was therefore, which mechanisms are responsible for changes in viscosity upon increasing the total protein concentration, varying the casein/whey protein ratio, and heating. In order to answer this question, we investigated the viscosity of un-heated and heated milk protein concentrates (3-14% total protein) and three casein/whey protein ratios (85:15, 92:8, 98:2). It turned out that a 37% whey protein depletion was sufficient to 1) inhibit the impact of the casein micelles' repulsive forces on the viscosity; 2) achieve the highest polydispersity and, therefore, particle packing density of all concentrates; and 3) allow up to 95% whey protein denaturation without changing the viscosity of the concentrates after heating.

Keywords: viscosity; whey protein denaturation; protein aggregates; polydispersity; microfiltration; ultrafiltration

3.3.1. Introduction

In the cheese manufacture it is common to increase the protein content of the vat milk for a higher cheese yield. For fresh and hard cheese, a high casein concentration is needed, whereas for quark a high total protein concentration and a high degree of whey protein denaturation are desired at the same time. The protein concentration and composition can be varied via micro- (MF) and ultrafiltration (UF) (Kelly et al., 2008). An alternative to filtration processes is enriching the vat milk with protein powders comprised of milk protein or micellar casein (Guinee et al., 2006).

The idea of the present study is to produce milk protein powders as base for fresh/hard cheese and quark production already having the optimal composition for manufacturing each of those cheese types. This would require only one rehydration step, which can be performed continuously e.g., with rotor/stator shearing devices or high pressure homogenizer (Warncke and Kulozik, 2021, 2020) and no further unit operations like protein adjustment or heating; consequently, the production time can be reduced. The powder for fresh/hard

[5] Adaptions refer to formatting issues: e.g., numbering of sections, figures, tables, and equations; abbreviations, manufacturer specifications, axis labeling, figure captions, and style of citation. References are listed at the end of this thesis, combined with the references of the other publications, to avoid redundancies.

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cheese should have a high casein and a low whey protein concentration. Considering powder for quark production, it optimally has a high total protein concentration, whereby the whey proteins are denatured and bound to the casein micelles. Another benefit of this process is that the powder manufacturer can remove the whey proteins in advance making the sweet whey collection and purification for the consumer unnecessary. However, concentration impacts inevitably the concentrate's viscosity—particularly after heat treatment. Our research question was, therefore, which macroscopic mechanisms are responsible for changes in viscosity upon increasing the total protein concentration, varying the casein/whey protein ratio, and heating.

On the one hand, the apparent viscosity depends on the volume fraction of casein micelles, native and denatured serum proteins. On the other hand, the apparent viscosity of the dispersion is high if the inherent viscosity of the continuous phase (lactose-dependent) is high as well (Anema et al., 2004; Jeurnink and de Kruif, 1993; Snoeren et al., 1982). The protein volume fraction can be raised by increasing the total protein concentration, which in turn, alters the protein voluminosity, or in other words, the protein hydration ratio (Nöbel et al., 2012). Furthermore, pH (Anema and Creamer, 1993; Snoeren et al., 1984a; van Hooydonk et al., 1986), casein genetic variants (Anema and Creamer, 1993), colloidal calcium phosphate levels (Aoki and Kako, 1983; Snoeren et al., 1984b), as well as temperature (Snoeren et al., 1984b) affect the proteins' voluminosity. When milk is heat-treated, whey proteins can either bind to the κ-casein on the micelle surface increasing the micelle size, or to serum κ-casein. In presence of whey proteins, this effect is predominantly based on the formation of disulfide bonds upon unfolding of β-lactoglobulin rendering a sulfhydryl-group reactive, with contributions of other types of chemical bonds (Corredig and Dalgleish, 1996; Jang and Swaisgood, 1990; Smits and van Brouwershaven, 1980). The latter ones become more decisive as the whey protein ratio is decreased. In addition, whey proteins can interact with themselves forming small, dispersed aggregates (Nair et al., 2013). Consequently, the voluminosity and hence, volume fraction of the whey proteins increase as well (Langley and Temple, 1985; Snoeren et al., 1984b, 1982). Since milk is concentrated and heat-treated in this study, the protein concentration and casein/whey protein ratio must be combined in such way that the proteins' voluminosity is kept low so that the milk protein concentrate remains fluent after heating.

There are different possibilities to vary the casein/whey protein ratio: One way is to concentrate with UF and MF first and to dilute the concentrate with UF permeate afterwards (Renhe and Corredig, 2018). Doing so, two- and fourfold-concentrated, diluted UF and MF retentates showed similar viscosities at the same volume fractions. The authors concluded that a whey protein removal does not affect the viscosity. Another way to adjust the casein/whey protein ratio is MF in diafiltration (DF) mode. Sauer et al. (2012) showed that a 65% whey protein-depleted milk protein concentrate had a lower viscosity than a 95% whey protein-depleted one at the same casein concentration. It is important to note that in this process, the casein concentration was adjusted, whereby, the volume fraction varied. Despite valuable insights into the rheological properties of milk protein concentrates, the results of the cited studies hardly can be directly compared because they refer either to the same volume fraction or to the same casein concentration. To allow direct comparison of UF and MF concentrates over a wide total protein range at the same volume fraction, we used an adapted filtration process (MF in DF mode and a subsequent UF) in this study.

Numerous studies focus on only one main effect on the viscosity of milk protein concentrates. Among these are the total solid content (Anema et al., 2014; Bienvenue et al., 2003a; Solanki and Rizvi, 2001), the casein/whey protein ratio (Renhe and Corredig, 2018; Sauer et al., 2012), and the whey protein denaturation (Anema et al., 2014; Bienvenue et al., 2003a; Kessler and Beyer, 1991). However, these studies do not clarify

whether and how the factors total protein concentration, casein/whey protein ratio and whey protein denaturation interact with each other and whether the main effects or the factor interactions affect the apparent viscosity in the first place. Thus, the objective of this study was to investigate the three main effects total protein concentration, casein/whey protein ratio, and whey protein denaturation as well as their interaction-dependent effects on the viscosity.

The viscosity increase observed for unheated and heated milk protein concentrates with increasing casein/whey protein ratios can be explained based on two effects: First, the interaction of casein micelles due to electrostatic forces and steric effects (de Kruif and May, 1991). In case of unheated concentrates, casein micelles are affected by repulsive forces in consequence of the negative charge of κ-caseins located on the micelle surface (Dalgleish, 2007). Regarding heated concentrates, denatured whey proteins associated with the κ-caseins interact predominantly (Dalgleish and Corredig, 2012). The second effect is the geometrical arrangement of the proteins and protein complexes differing in size. Polydispersity increases the volume fraction because small particles fill the gaps between bigger ones (Schaertl and Sillescu, 1994). Consequently, casein/whey protein complexes and whey protein aggregates forming during heating induce a higher viscosity. Thus, we hypothesize that the viscosity can be adapted by taking these effects into account.

Therefore, we investigated the viscosity of three milk protein concentrates of different casein/whey protein ratios (85:15, 92:8, 98:2) for total protein concentrations between 3.6% and 18.1%. For 0% whey protein depletion, skim milk was simply concentrated (UF) without diafiltration (no whey protein removal). For 37% and 88% whey protein depletion, skim milk was filtered (MF) in diafiltration mode first and subsequently concentrated using UF in order to keep the casein/whey protein ratio constant over the whole total protein range. This approach allowed the comparison between three different compositions at the same volume fraction. Moreover, the impact of whey protein denaturation on the rheological properties of the milk protein concentrates was assessed. Finally, we performed statistical modeling. This allows predicting the viscosity of unheated and heated milk protein concentrates as a function of different concentrations and casein/whey protein ratios.

3.3.2. Materials and Methods

3.3.2.1. Milk protein concentrate production

In this study, three milk protein concentrates with different total protein concentrations and casein/whey protein ratios were produced. Figure 3.16 shows the related process scheme.

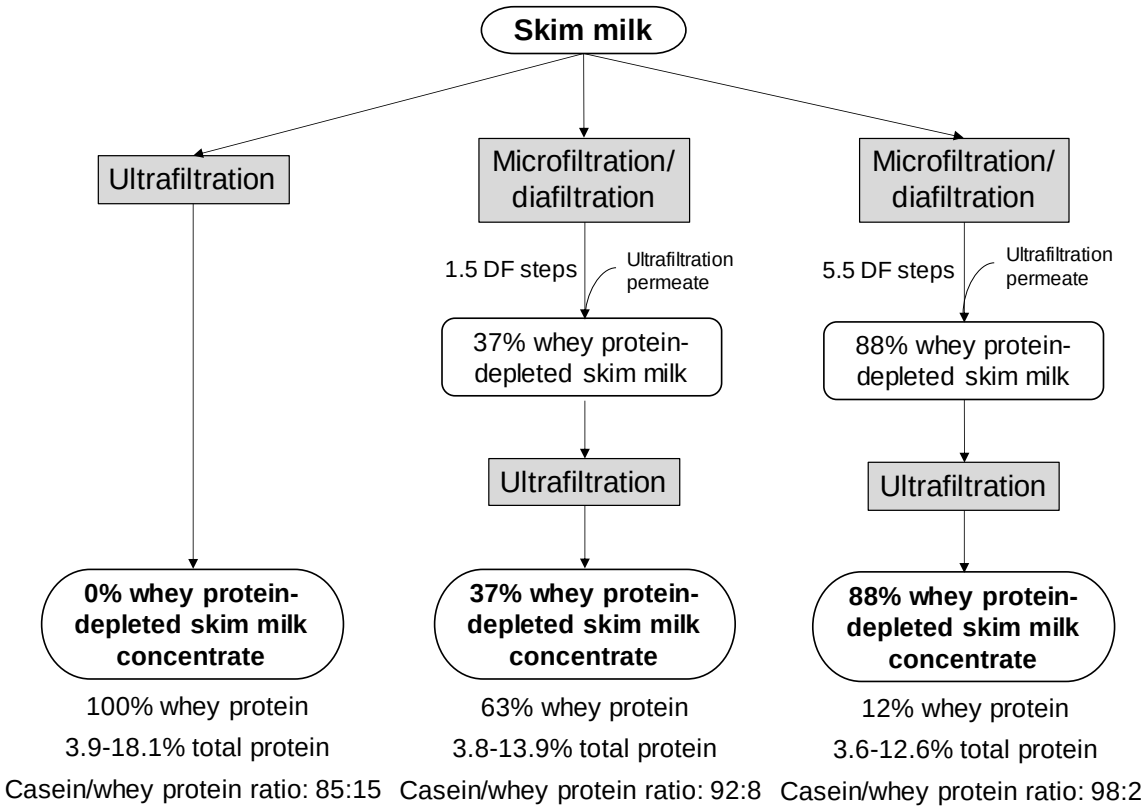

Figure 3.16 Process scheme for milk protein concentrate production.

For the experiments, pasteurized skim milk (74 °C, 28 s) was purchased from the local dairy (Molkerei Weihenstephan GmbH & Co. KG, Freising, Germany). For the filtration steps, we used polymeric membranes (Suspended Screen TFF Cassettes with Supor Membrane P/N PSM10C11 (MF) and T-Series TFF Cassettes with Omega™ Membrane (UF), Pall Corporation, New York, USA) with molecular weight cut-offs of 0.1 μm and 10 kDa, respectively. The filtration temperature was 50 ± 1 °C. Milk was UF-concentrated to 18.1% total protein (protein concentration factor CF = 4.5) to produce a protein concentrate containing 100% of the whey proteins. For 37% whey protein depletion, milk underwent 1.5 diafiltration (DF) steps using milk serum (UF permeate) as DF medium, before getting UF-concentrated to 13.9% total protein. For 88% whey protein depletion, 5.5 DF steps were necessary prior to UF concentration to CF 3.4 from 3.6% (after DF) to 12.6% total protein. Samples for the analysis were taken in intervals during UF concentration. They were immediately cooled on ice and stored overnight at 4 ± 1 °C. All filtrations were performed in duplicate.

Casein and whey protein content (according to Dumpler et al., 2017) and lactose content (according to Schmitz-Schug, 2013) were determined by reversed-phase high performance liquid chromatography (RP-HPLC). Figure 3.17 shows the casein and whey protein content as a function of the total protein concentration.

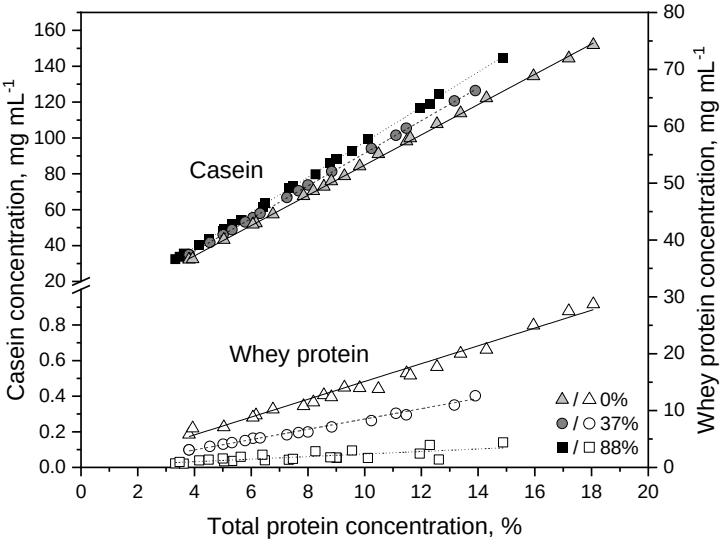

Figure 3.17 Casein (filled symbols) and whey protein concentrations (empty symbols) of 0, 37, and 88% whey protein-depleted concentrates.

Casein as well as whey protein concentration increased linearly with increasing total protein concentration. The casein content increased slightly with increasing DF steps; however, these differences were insignificant ($P \leq 0.1$). Regarding the whey protein content, the 88% whey protein-depleted concentrate was significantly lower than the 37%-depleted one ($P \leq 0.01$) and this in turn, was significantly lower than the 0% whey protein-depleted concentrate ($P \leq 0.1$). The lactose concentration was 47.2 ± 7.0 mg mL^{-1} for all concentrates.

3.3.2.2. Heat treatment

Samples were heated to induce whey protein denaturation and aggregation. 10 mL of the samples were filled in 25 mL round-bottom flasks (D = 39.0 mm) and heated (80 °C for 30 min) in a water bath under steady stirring with a magnetic stir bar (L = 30.5 mm, D = 6.2 mm). Afterwards, the samples were immediately cooled on ice to ~4 °C. The degree of whey protein denaturation was determined by RP-HPLC (according to Dumpler et al., 2017). Therefore, we precipitated denatured whey proteins and caseins at pH 4.6 in advance. It needs to be considered, that the pellet size of the precipitated proteins increases and the volume of the supernatant decreases with increasing casein/whey protein ratio. As a result, the native whey proteins are concentrated in the soluble phase. Consequently, we took the voluminosity of the casein micelles for the calculation of the degree of whey protein denaturation into account. We calculated the correction factor (CF) and the degree of whey protein denaturation (DD) as follows and assumed a value of 4.4 cm^3 g^{-1} for the casein voluminosity (Broyard and Gaucheron, 2015).

$$CF = \frac{100 - 4.4\ cm^3 g^{-1} \cdot c_{Total\ protein} \cdot Casein\ ratio}{100} \tag{3.9}$$

$$DD\ [\%] = \left(1 - \frac{c_{Native\ whey\ protein} \cdot CF}{c_{Whey\ protein}}\right) \cdot 100 \tag{3.10}$$

3.3.2.3. Ultracentrifugation and particle size measurement

Ultracentrifugation was performed to separate the soluble fraction containing whey proteins, serum casein, whey protein aggregates, and soluble casein/whey protein aggregates from the sedimentable fraction containing casein micelles and casein/whey protein complexes. This allows detecting the formation of soluble aggregates after heating. Particle size changes in the soluble phase of unheated and heated samples were measured by dynamic light scattering using a Malvern Zetasizer Nano ZS (Malvern Instruments GmbH, Herrenberg, Germany). Suitable centrifugation conditions and dilutions were determined in preliminary experiments (not shown). Samples were diluted 3 to 7 times depending on the protein content in order to reach a total protein concentration of 1.2% to 2.5% in the corresponding UF permeate. Because we adjusted the total protein concentration of all samples in the corresponding UF permeate prior to the measurements, no differences in the measurement results for unconcentrated and concentrated, diluted samples were observed. Therefore, it was reasonable to use average values of 10 measurements of eight individual samples (eight unheated and eight heated) over the entire range of total protein concentrations. 1.5 mL of the diluted samples were ultracentrifuged at 27 500 rpm (84 700 × *g*) for 90 min at 25 °C in 1.5 mL Eppendorf tubes. For this, a Thermo Scientific Sorvall wX80+ equipped with a Fiberlite F50L-24x1.5 rotor (Thermo Fisher Scientific, Waltham, USA) was used. Proteins found in the supernatant were considered as soluble. 1 mL of the clear supernatant was carefully removed and filtered through a 0.45 µm syringe filter into the cuvette used for particle size measurement. After an equilibration time of 2 min, samples were measured 10 times in 173° backscatter mode at 20 °C for 1 min each.

3.3.2.4. Rheological measurements

To evaluate the influence of the total protein concentration, the casein/whey protein ratio, and the degree of whey protein denaturation on the rheological behavior of milk protein concentrates, we used the MCR 302 rheometer (Anton Paar GmbH, Graz, Austria) equipped with a cone/plate geometry (2°, d = 50 mm). The sample volume was 1.15 mL and the measuring temperature was 20 °C. The sample was pre-sheared at 500 s^{-1} for 1 min before resting for 15 s to allow the system to equilibrate. The shear stress was monitored over a shear ramp from 1 to 1000 s^{-1} within 9 min following Anema et al. (2014).

As is the custom, flow curves were fitted using the power law model (3.11) using the software Rheo Compass (version 1.22, Anton Paar GmbH, Graz, Austria). The model describes the apparent viscosity η (Pa s) of a non-Newtonian fluid like milk concentrate as a function of the consistency coefficient K (Pa s^n) , the shear rate $\dot{\gamma}$ (s^{-1}), and the flow behavior index n (-) (Bienvenue et al., 2003b; Dahbi et al., 2010; Fernández-Martan, 1972; Nöbel et al., 2012; Snoeren et al., 1982; Vélez-Ruiz and Barbosa-Cánovas, 1998).

$$\eta = K \cdot \dot{\gamma}^{n-1} \tag{3.11}$$

If $n < 1$, the solution behaves shear-thinning, if $n = 1$, it is Newtonian and if $n > 1$, it behaves shear-thickening.

3.3.2.5. Statistical analyses and modeling

Origin 2020 (OriginLab Corporation, Northampton, United States) was used to plot graphs. RStudio, Inc., 2019 (version 1.2.5033, Boston, MA, United States) was used for statistical analysis and JMP® Pro (Version 14.1.0, SAS Institute Inc., Cary, USA) was used for statistical modeling. Statistical significances were evaluated using one-way analysis of variance (ANOVA) combined with Tukey's HSD post-hoc test. The calculated *P*-values and the respective significance levels are given in the text ($P \leq 0.001$, $P \leq 0.01$, $P \leq 0.05$, $P \leq 0.1$).

For the statistical modeling, we assumed a quadratic model in form of Equation (3.12). This equation expresses the impact of n independent factors from x_i to x_n on the response y scaled by the intercept β_0, the partial regression coefficients for the factors, β_1, β_{11}, $\beta_{1\ldots n}$, and the residuals ξ.

$$y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j + \xi \quad (3.12)$$

2/3 of the observations (54) were used for modeling and validated with the remaining 1/3 of the observations (26). The modeling procedure followed Kieferle and Kulozik (2021) and is described in detail elsewhere. Stepwise regression was applied and the significance of the model effects was evaluated based on t-statistics ($\alpha = 0.05\%$). Based on the corrected Akaike information criterion (AICc) and under consideration of heredity restriction (Akaike, 1974; Spiess and Neumeyer, 2010), the best model with a maximum of 10 terms was chosen. The heredity restriction prohibits the removal of non-significant factors from the model when interactions including these factors are significant.

The filtrations were performed in duplicate. Each data point represents one single measurement. All curves contain data points from two independent samples.

3.3.3. Results and Discussion

3.3.3.1. Relationship between viscosity, particle size, and casein/whey protein ratio

To elucidate the impact of heat treatment, casein/whey protein ratio and protein concentration on rheological properties of milk protein concentrates used for cheese production, we performed rheological measurements, quantified the degree of whey protein denaturation, and determined particle sizes.

3.3.3.1.1. Impact of the total protein concentration on the apparent viscosity

Figure 3.18 illustrates the apparent viscosity as a function of the total protein concentration at 1000 s^{-1}. Tab. S1 in the supplementary material shows the related consistency coefficients *K* and the flow behavior indexes *n* calculated using the power law model. For all concentrates, we observed that the viscosity increased exponentially with increasing total protein concentration.

Having a look on the unheated samples first, we observed that the viscosity of the unheated 0% and 37% whey protein depleted concentrates behaved similarly as it increased exponentially from ~2 mPa s at 3.8% total protein to ~10 mPa s at 13.6% total protein (Figure 3.18 A, B). The viscosity of the 88% whey protein-depleted concentrates differed noticeably from the others (Figure 3.18 C). It increased exponentially from 2.2 mPa s at 3.5% total protein concentration to 60.6 mPa s at 12.3%.

This has two reasons: The hydration and the net negative charge of the casein micelles. On the one hand, the reduced free water increases the packing density of the caseins with an increase in total protein concentration, although their volume fraction remains the same. On the other hand, the distance between the micelles decreases, which in turn leads to more intense electrostatic repulsion. Therefore, the same-charged particles repel each other and thus, change their flow direction. As a result, the flow resistance of the suspending medium increases (Ibanoğlu, 2002). At higher protein concentrations, the hairy layer of the casein micelles is compressed and interpenetrated, so their motions depend on hydrodynamic and inter-particle interactions (Nair et al., 2013).

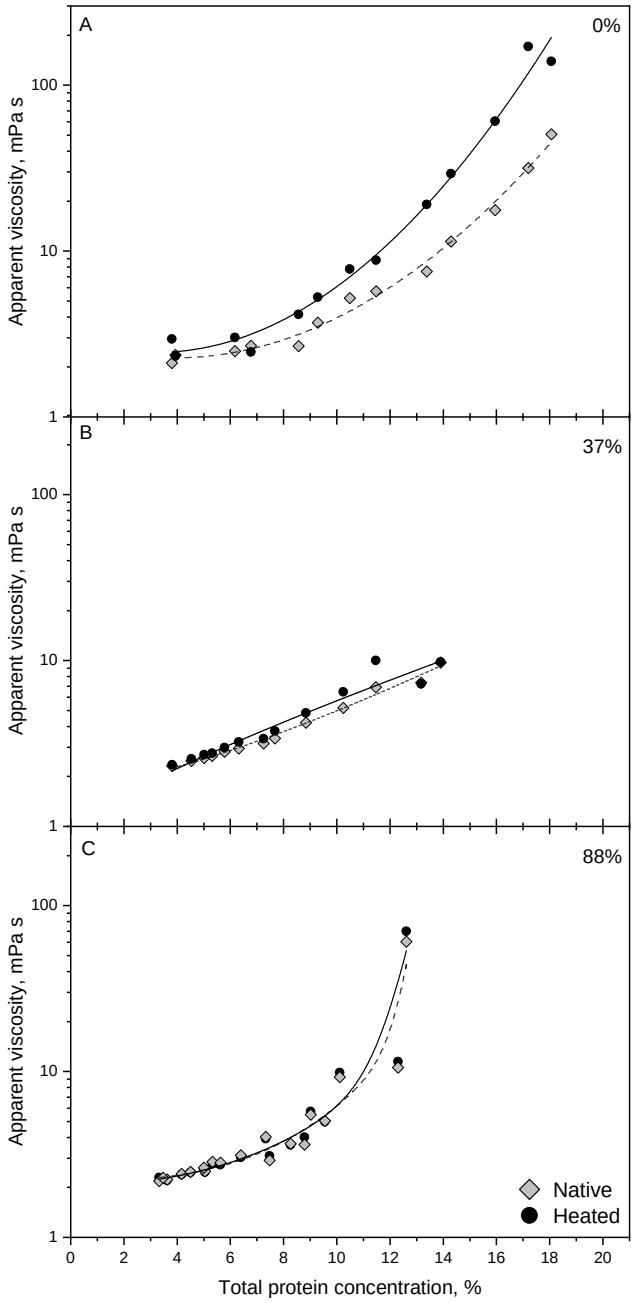

Figure 3.18 Apparent viscosity ($\dot{\gamma}$=1000 s^{-1}, ϑ=20°C) of unheated and heated (ϑ=80°C, t=30 min) 0 (A), 37 (B), and 88% (C) whey protein-depleted concentrates. Each data point represents one measurement. Each curve is based on two independent replicates, where each point represents one measurement.

The flow behavior of casein micelles dispersed in milk permeate at native pH can be described as for hard spheres (de Kruif, 1998; Horne, 2003). The viscosity can be expressed by the model of Krieger and Dougherty (1959), which takes the increasing particle interactions in concentrated suspensions into account. With protein complex formation (serum aggregates as well as casein/whey protein complexes) as it occurs on heating, the particle polydispersity in a concentrate increases compared to the unheated one. The presence of casein/whey protein complexes and soluble whey protein affect the rheological properties due to the increased voluminosity (Anema et al., 2014; Langley and Temple, 1985; Snoeren et al., 1984b, 1982) as we observed for the 0% whey protein-depleted sample. After heating, the viscosity increased markedly (Figure 3.18). The extent of complexation or aggregation depend on the protein concentration and the casein/whey protein ratio. Therefore, we investigated how these factors affect the whey protein denaturation and the particle size and this in turn, the viscosity, to explain the rheology of the heated samples.

3.3.3.1.2. Relationship between the total protein concentration and the degree of whey protein denaturation

Figure 3.19 shows the degree of whey protein denaturation as a function of total protein concentration for the 0%, 37%, and 88% whey protein-depleted concentrates. At low total protein concentrations, i.e., between 3% and 7%, the degree of whey protein denaturation of all samples was lowest and increased strongly with increasing total protein concentration. Once the total protein concentration was doubled in comparison to fresh milk (> 7% total protein concentration), the denaturation of all samples reached the maximum possible degree of ~95%.

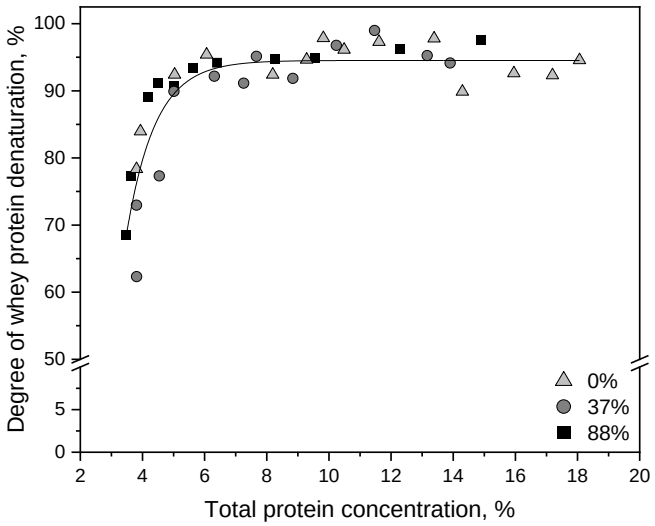

Figure 3.19 Degree of whey protein denaturation as a function of total protein concentration of the concentrates after heating (80 °C for 30 min). The degree of whey protein denaturation of the pasteurized skim milk is 5.36 ± 0.21% (average ± sd).

The sharp increase in the degree of whey protein denaturation at lower total protein concentrations can be explained based on the collision rate of whey proteins, which increases with increasing total protein concentrations due to the higher number of whey protein molecules. As a consequence, the aggregation rate increases and the denaturation reaction accelerates until the total protein concentration reaches 7% (Anema et al., 2006; Wolz et al., 2016). Another reason is that the lactose/protein ratio decreases with an increasing total

protein concentration. In the presence of sugars, whey proteins prefer the associated form to avoid unfavorable water-protein interactions (Arakawa and Timasheff, 1982; Timasheff, 2002). Thus, lactose has a protective effect on β-lactoglobulin against denaturation as long as the lactose/protein ratio is high enough (Bernal and Jelen, 1985; Garrett et al., 1988; Jou and Harper, 1996; Plock et al., 1998; Spiegel, 1999). Finally, changes in the ionic strength and the pH are known to affect the protein properties (e.g., zeta potential) and aggregation behavior (Engelhardt et al., 2013; Schmitt et al., 2007). However, these effects are presumably not pronounced enough to induce significant changes in the degree of whey protein denaturation in our case. Interestingly, there is no difference between the 0%, 37%, and 88% whey protein-depleted concentrates. The reason is that the collision probability of the whey proteins with either casein micelles or other whey proteins was similar in all samples at the same total protein concentration due to the unaltered amount of reactive binding sites (other whey proteins as well as casein micelles).

Although, the degree of whey protein denaturation is similar for all casein/whey protein ratios, their viscosities differ. The degree of whey protein denaturation determined by pH 4.6 precipitation reveals how much whey protein is unfold and denatured, but it does not consider the size of formed aggregates, which seems to be the decisive factor influencing the viscosity after heating.

3.3.3.1.3. Changes of the particle size of concentrates differing in their whey protein ratio upon heating

In order to assess the formation of heat-induced whey protein aggregates and soluble casein/whey protein aggregates, particle sizes in the supernatants after ultracentrifugation (84 700 × g for 90 min at 25 °C) were measured. Furthermore, the modality of the curves provides insights into the polydispersity of the concentrates. Figure 3.20 shows the cumulative particle size distributions of the supernatants of the unheated (A) and heated (B) samples measured by dynamic light scattering.

Having a look at the unheated samples first, the particle sizes in the supernatants increased with increasing casein/whey protein ratio. In the 88% whey protein-depleted concentrate the concentration of soluble whey proteins was lowest, and the concentration of casein highest compared to the other samples. Due to the fact that these particles are larger than whey proteins, we assumed that the larger particles are casein fragments dissociated from the casein micelle. As a reason, temperature- or pH-dependent dissociation can be excluded, because samples were warmed to room temperature before dilution and the pH of the dilution medium was 6.7, too. Our results indicate that the milk salts need to equilibrate between UF or MF retentates and UF permeate—although it is said to keep the milk salt milieu constant—and that casein particles dissociate from the micelles. It is already known that diluting casein micelles in water or diafiltrating against water leads to dissociation due to loss in calcium and phosphate levels (McKenna, 2000). The fact that this occurs in UF permeate as well, is an interesting observation, which is, to our knowledge, not reported in the literature so far. Pursuing studies in this field are necessary to confirm our assumption.

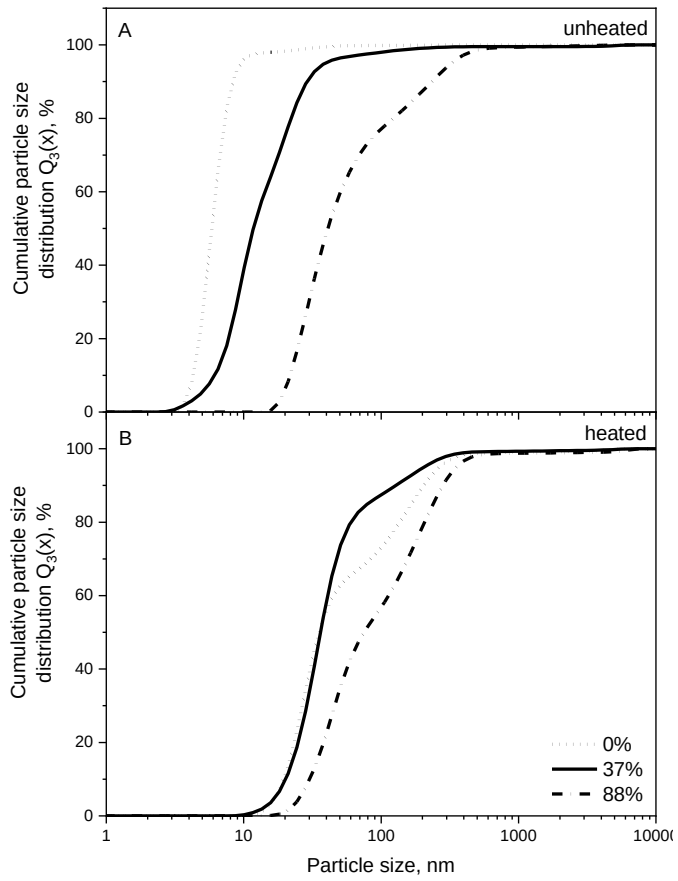

Figure 3.20 Cumulative particle size distribution $Q_3(x)$ of unheated (A) and heated (B) concentrates' supernatants after ultracentrifugation (84 700 × *g* for 90 min at 25 °C).

When milk is heated, the particle sizes change compared to unheated milk due to aggregate formation. Either casein/whey protein complexes or soluble whey protein and κ-/α_s-casein/whey protein complexes form (Renhe and Corredig, 2018). The particle size distributions of the heated samples (Figure 3.20 B) indicate those soluble complexes as all distributions shift from smaller to larger particle sizes upon heating. Casein micelle/whey protein complexes have a 15% larger volume than native casein micelles (Anema et al., 2004) and sediment under the applied centrifugation conditions. We observed the largest particle size shift for the 0% whey protein-depleted concentrates. The high ratio of whey proteins implies their high concentration in the serum phase. This in turn facilitates collision between the molecules and whey protein aggregate growth. In case of a reduced whey protein content—like in the 37% and 88% whey protein-depleted concentrates—the whey protein aggregate growth is inhibited due to the fact that less reactive sulfhydryl-groups from β-lactoglobulin are available for generating disulfide bonds (McKenzie et al., 1971; Smits and van Brouwershaven, 1980), also for the formation of aggregates between κ-casein at the casein micelle surface and β-lactoglobulin. Consequently, smaller aggregates form. This manifests itself in the reduced diameter ($d_{90,3}$) of the 37%-depleted samples (~100 nm) compared to the 0%-depleted ones (~110 nm). In all cases, the distribution was bimodal after heating indicating polydispersity in the supernatant.

Summing up the results presented in this section, an increase in total protein concentration leads to a significant increase in apparent viscosity and whey protein denaturation. The particle size increases upon

heating in general. However, there seems to be an interaction with the whey protein content because the particle size of 0% whey protein-depleted concentrates lies between that of 37% and 88% whey protein-depleted concentrates. In order to clarify the nature of this and other interrelationships of influencing factors, we applied statistical modelling.

3.3.3.2. Clarification of interrelationships between factors determining the concentrate's apparent viscosity by statistical modeling

During milk processing, a high viscosity can lead to reduced flow rates, high pressure drops, decreased turbulence, and severe fouling in heating operations (Bienvenue et al., 2003b). A model allowing the prediction of the viscosity for unheated and heated concentrates of different compositions and concentrations can help to optimize process designs and to avoid the effects mentioned before. Therefore, we performed multiple non-linear regression modeling to express the logarithmic apparent viscosity as a function of total protein concentration, whey protein ratio (% whey protein of the total protein concentration), and degree of whey protein denaturation, which was not considered in Figure 3.18. The logarithmic apparent viscosity delivered a linear relation and therefore, a better goodness-of-fit than calculating with the non-logarithmic one. The model is characterized in Table 3.7.

Table 3.7 Parameter estimates and corresponding *P*-values defining the statistical model for the logarithmic apparent viscosity (mPa s), where β_0 is the intercept, c_{TP} (%) is the total protein concentration, c_{WP} (%) is the whey protein ratio (% whey protein of the total protein concentration) and DD (%) is the degree of whey protein denaturation. Asterisks mark level of significance (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$). Non-significant terms remain in the model due to the heredity restriction. The correlation coefficient R^2 was 0.89.

Model effect	Estimate	*P*-value
β_0	0.2689014	0.0207 *
c_{TP}	0.0210669	0.4550
c_{WP}	- 0.015072	0.3252
DD	0.0058699	0.0757
$c_{TP} * c_{WP}$	- 0.002702	0.0047 **
$c_{TP} *$ DD	0.000391	0.0013 **
$c_{TP} * c_{TP}$	0.0042392	0.0196 *
$c_{WP} * c_{WP}$	0.0015787	0.0374 *
DD * DD	$- 8.963 \times 10^{-5}$	0.0273 *

It turned out that all factors (c_{TP}, c_{WP}, and DD) are contributing to significant interactions. Thus, it is indispensable to take each factor into account for predicting the viscosity at 1000 s^{-1}. The total protein concentration c_{TP} interacted with the whey protein ratio c_{WP} as well as with the degree of whey protein denaturation DD. The interaction of the whey protein ratio and the degree of whey protein denaturation is not considered in the model. The reason is that the degree of whey protein denaturation depends on the total protein concentration but is independent of the whey protein ratio as shown in section 3.3.3.1. The interactions between the total protein concentration and the whey protein ratio and between the total protein concentration and the degree of whey protein denaturation affect the viscosity most. As demonstrated in Figure 3.18 and Figure 3.21, the apparent viscosity increases strongest with increasing total protein concentration and decreasing whey protein

ratio (or increasing casein/whey protein ratio). Solanki and Rizvi (2001) observed the same trends for MF retentates. Milk was 2 to 8 times MF-concentrated, whereby, the casein/whey protein ratio increased. The concentrates showed an increased shear stress with increasing concentration factor or in other words, with increasing total protein concentration and casein/whey protein ratio as well. The interaction of total protein concentration and degree of whey protein denaturation is in agreement with the results of Wolz et al. (2016), Kessler and Beyer (1991), and Pierre et al. (1977) who focused on whey protein concentrate solutions, casein/whey protein solutions with a ratio of 40/60, and UF concentrates, respectively. All authors observed an increased degree of whey protein denaturation with increasing total protein concentration.

Figure 3.21 A compares the logarithmic viscosity observed during the experiments with the values determined applying the model defined in Table 3.7. With a correlation coefficient R^2 of 0.89, the representation of the data by the model is very good. The histogram and normal Q-Q plot of the residuals indicated their normal distribution (not shown). Up to a logarithmic apparent viscosity of 1.1 mPa s (corresponds to ~19 mPa s, non-logarithmic), the model fits quite well. Exceeding a value of 1.1 mPa s, the predictability of the model becomes less reliable due to the discrepancies between the high viscosities of the 88% whey protein-depleted concentrates and the very low viscosities of the 37% whey protein-depleted samples observed at their highest protein concentrations.

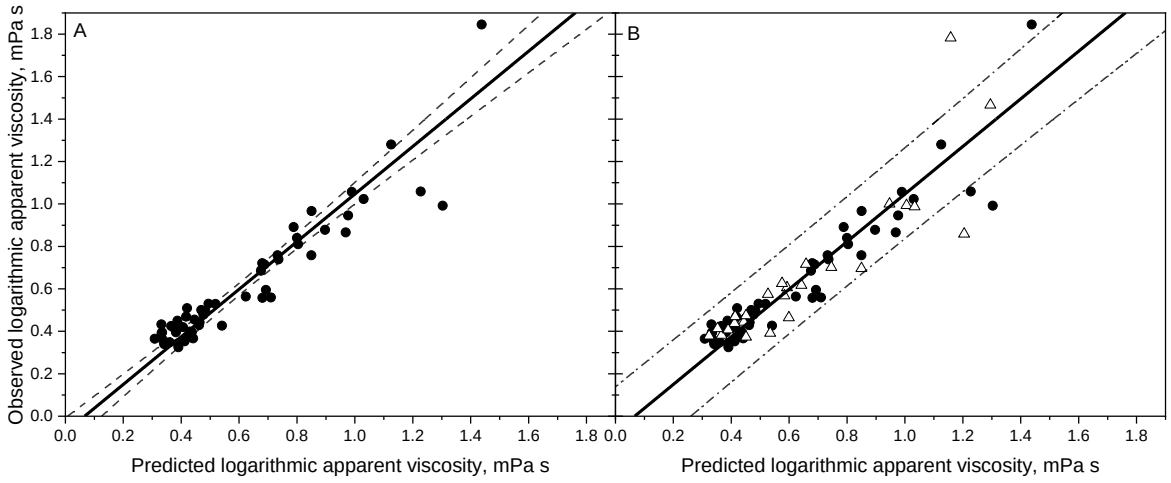

Figure 3.21 Parity plot of the model with 95% confidence interval (dashed lines) (A). Validation of the statistical model (black circles) using observed data not utilized for modeling (white triangles). The dashed/dotted lines indicate the 95% prediction interval of the model. The correlation coefficient R^2 of the model was 0.89.

In order to demonstrate that the model is not only valid for the modeling data but has a certain degree of general validity, we inserted independent data not used for the modeling and checked them on conformity. This validation is presented in Figure 3.21 B. It turned out that 92.3% of the observed viscosities of the independent data (white triangles) lie within the 95% prediction interval and can be described with the model. The two data points from the independent data lying beyond the prediction interval can be traced to the major differences between the 37 and 88% whey protein-depleted concentrates at their highest protein concentrations as well. Area/dot plots of the modelled and observed apparent viscosities of the unheated and heated concentrates as a function of total protein concentration and whey protein ratio are appended as supplementary material (Figure S1).

In summary, all factors, namely the total protein concentration, the whey protein ratio, and the degree of whey protein denaturation interact and thus, affect the concentrate's apparent viscosity. In order to cover the underlying mechanisms, we derived a model representation of the particle interactions in concentrated, whey protein-depleted, and heated concentrates.

3.3.3.3. Model representation of the mechanisms underlying changes in the viscosity upon concentration, whey protein depletion, and heating

Figure 3.22 illustrates schematically the casein/whey protein ratio-, particle size- and polydispersity-dependent viscosity of the 0%, 37%, and 88% whey protein-depleted concentrates to better visualize the effects mentioned before.

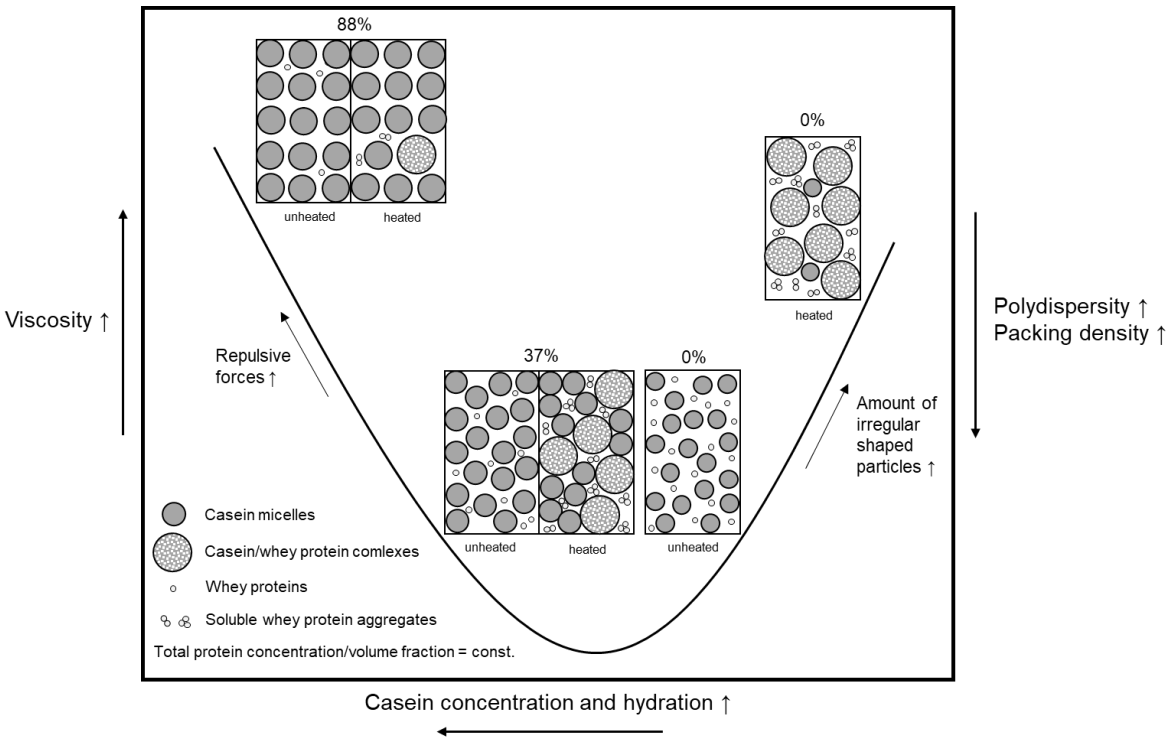

Figure 3.22 Model representation of the mechanisms underlying changes in the viscosity upon concentrating and heating at a constant total protein concentration/volume fraction of 0, 37, and 88% whey protein-depleted milk protein concentrates. Illustrations of the proteins are schematic and are not intended to meet the scale.

The higher the degree of polydispersity is, the higher is the particle packing density in dispersions (Schaertl and Sillescu, 1994), because small particles (here, whey proteins or soluble aggregates) fill the gaps between large particles (here, native casein micelles or casein/whey protein complexes). This in turn, decreases the viscosity. Either a higher casein/whey protein ratio and therefore, a lower amount of casein/whey protein complexes after heating or a lower casein/whey protein ratio without heating go hand in hand with a higher polydispersity. Therefore, unheated and heated 37% and unheated 0% whey protein-depleted concentrates show the lowest viscosities (Figure 3.22). This effect is compensated in the heated 0% whey protein-depleted samples upon heating, due to its extensive particle growth: Casein/whey protein complexes cannot be considered round spheres anymore; irregular gaps may not be filled out perfectly by the whey protein aggregates resulting in a lower particle packing density. Consequently, the viscosity increases.

The smallest shift in particle size was observed for the 88% whey protein-depleted samples ($d_{50,3}$ increased from ~40 to 70 nm, Figure 3.20) due to the small impact of the relatively low amounts of whey proteins. Since the particle sizes of the heated 88% whey protein-depleted samples were almost not altered, the casein micelles can be still considered as same-sized spheres, which have a lower polydispersity and hence, particle packing density. However, considering the nature of native casein micelles (net negative charge and hydration), the unheated and heated 88% whey protein-depleted concentrates showed high viscosities at high total protein concentrations as well.

Heating led to whey protein denaturation and increased particle sizes as shown before. Heat had the strongest effect on the particle sizes of the 0% whey protein-depleted concentrates as its particle growth in the supernatant after heating was greatest. Therefore, we expected the most pronounced changes in the apparent viscosity after heating for these samples. It turned out that the heated 0%-depleted samples became more viscous compared to the unheated ones (Figure 3.18 A). This can be explained as follows: Free, non-aggregated whey proteins enabled a closer packing of the protein volume fraction. Heating induced larger, less round-shaped aggregates. Consequently, the packing density decreased and the viscosity increased (Santamaría-Holek and Mendoza, 2010). We expected, based on the particle size results, that heat has no significant ($P \leq 0.1$) impact on the viscosity of the 37% and the 88% whey protein depleted concentrates, which we can confirm (Figure 3.18 B, C). Here, the changes in particle size and packing density after heating were not pronounced enough to have an impact on the viscosity. The viscosity of 37% whey protein-depleted concentrates increased nearly linearly with increasing total protein concentration up to ~10 mPa s at 14% total protein concentration (Figure 3.18 B). However, the viscosity of the 88% whey protein-depleted concentrates is still higher at total protein concentrations above 10% than the viscosity of the heated 0%-depleted concentrates. With increasing polydispersity (88% < 0% < 37%) the packing density increases (Schaertl and Sillescu, 1994) and the viscosity at a given particle concentration decreases. Therefore, the 37% whey protein-depleted concentrates had a lower viscosity at high total protein concentrations than the 0%-depleted ones.

3.3.4. Conclusions

This study demonstrates in which way heat treatment, variation of protein concentration, and casein/whey protein ratio affect the rheological properties of milk protein concentrates.

Based on experimental data, we confirmed our hypothesis that the viscosity can be adapted by taking into account the casein micelle/whey protein interactions and the geometrical arrangement of the proteins and protein complexes differing in size. Repulsive forces become more dominant as the whey protein content is reduced and the casein concentration increased, due to the casein micelles' high net negative charge, particularly at high total protein concentrations. The polydispersity alters upon changing the casein/whey protein ratio or upon heating when casein/whey protein complexes and soluble whey protein aggregates of different sizes form. A high polydispersity goes hand in hand with a high particle packing density, which in turn results in a reduced viscosity. We could show that a whey protein depletion of 37% was sufficient 1) to keep the repulsive forces between the casein micelles beyond the sphere of influence on the viscosity; 2) to achieve the highest polydispersity and particle packing density of all concentrates; and 3) to allow up to 95% whey protein denaturation without changing the viscosity of the concentrates after heating. Our results indicate that both heating and concentration are not limiting the viscosity inevitably. A heated concentrate remains fluent and easy to process when whey proteins were partially depleted—even at high total protein concentrations. If a

high casein concentration is desired, the maximum total protein concentrating should not exceed 9% to keep the casein micelles' hydration and their repulsive forces on an acceptable level at which the viscosity is still low and unaffected.

The statistical model allows predicting the apparent viscosity of milk protein concentrates of different total protein concentrations and casein/whey protein ratios and how pronounced the differences in viscosity might be after heating. This should help designing processes, such as the protein concentrate powder production used for fresh/hard cheese and quark, where the apparent viscosity is a limiting factor.

Acknowledgments: The authors thank Günther Unterbuchberger and Andreas Matyssek for their assistance in performing the filtration experiments. Moreover, we wish to express our gratitude to Eva-Maria Schmid, Claudia Hengst, Heidi Wohlschläger, Hermine Roßgoderer and Martin Hilz for help in conducting the analytical measurements. Special thanks go to Dr. Hannes Petermeier for his support in statistical analysis and to Eva Scheidler, Michael Reitmaier and Roland Schopf for their support and valuable discussions. This work was supported by our partner Arla Foods amba.

Supplementary material

Table S1 Calculated consistency coefficient K and flow behavior index n of the unheated and heated 0, 37 and 88% whey protein-depleted concentrates. The correlation coefficient R^2 of all power law fits was 0.99 ± 0.003. Each observation represents one singular measurement. Eight of the 15 observations of each casein/whey protein ratio are listed representatively with increasing total protein concentration.

	Total protein concentration [%]	Consistency coefficient K [mPa s^n]		Flow behavior index n [-]	
		Unheated	Heated	Unheated	Heated
0%	3.81	1.93	11.05	0.99	0.78
	6.17	2.86	4.22	0.96	0.94
	8.56	3.19	5.76	0.96	0.95
	10.49	7.04	12.91	0.96	0.93
	13.38	18.19	59.71	0.88	0.84
	15.95	58.82	1272.3	0.83	0.56
	17.20	160.08	1254.8	0.77	0.37
	18.07	975.62	1345.5	0.57	0.33
37%	3.81	1.64	1.68	1.08	1.04
	5.01	2.43	2.47	1.00	1.00
	6.32	3.02	2.74	0.99	1.02
	7.67	3.66	4.20	0.98	0.99
	8.84	4.84	4.64	0.99	1.02
	10.24	4.84	10.68	1.02	0.93
	11.47	10.20	15.70	0.95	0.94
	13.90	20.94	25.94	0.89	0.86
88%	3.47	3.03	1.96	0.94	1.02
	4.17	2.42	1.81	0.99	1.03

	5.05	4.20	3.71	0.90	0.92
	7.47	4.30	3.90	0.93	0.96
	8.79	5.03	5.96	0.96	0.94
	9.02	8.48	8.97	0.94	0.93
	10.12	19.45	22.28	0.90	0.89
	12.30	22.97	28.83	0.89	0.87

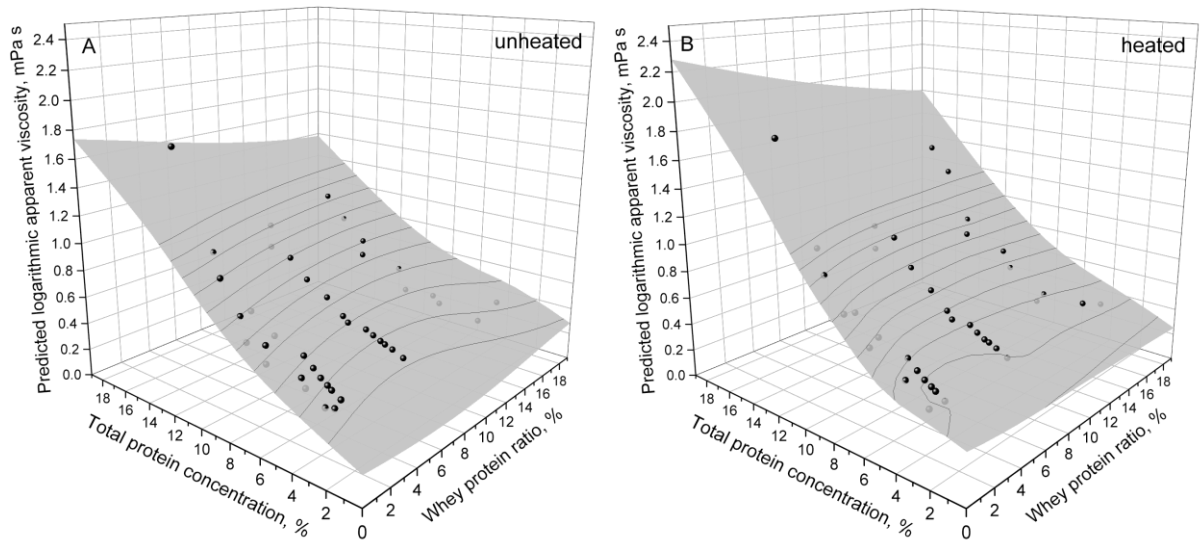

Figure S1 Area plots of the modelled apparent viscosities of the unheated (A) and heated (B) concentrates as a function of total protein concentration and whey protein ratio. Black spheres indicate the observed data. Contour lines visualize the values of the y-axis.

3.4. *Cold-renneted milk powders for cheese production: Impact of casein/whey protein ratio and heat on the gelling behavior of reconstituted rennet gels and on the survival rate of integrated lactic acid bacteria*

Summary and contribution of the doctoral candidate

During cheese manufacture, 70% of the starting amount of milk is converted to sweet whey, which is commonly purified to obtain the isolated whey proteins. In some parts of the world with lacking infrastructure for whey processing, the whey must be discarded and then potentially creates environmental burden. A novel concept to avoid whey disposal studied in this work is powder-based cheese manufacture with high yield due to an increased casein/whey protein ratio or the integration of the whey proteins in the cheese matrix. The aim was to avoid whey protein drainage by their prior removal or by their heat-induced structural integration in the curd.

The impact of the casein/whey protein ratio (86 : 14 and 98 : 2) and heat treatment (80 °C/30 min) on the gelling behavior of reconstituted rennet gels (meaning the reconstituted, gelled concentrates) and on the survival rate of integrated *Lactobacillus paracasei* ssp. *paracasei* F19 was investigated. The assessment criteria for the rennet gelation were curd firming rate, gel strength, and whey drainage. In addition, the amount of integrated whey proteins and the resulting cheese yield were evaluated. Furthermore, the viable cell count was determined after spray drying and during powder storage at 4, 10, and 20 °C during 103 days of storage.

We could show that heating had a positive effect on the gelation behavior of the reconstituted UF powder: The curd firming rate as well as the gel strength could be increased to higher values than the MF samples at 25% total solids. The higher serum holding capacity of the curd makes this powder suitable for cheese types with higher moisture contents. For cheeses with a lower moisture content, the MF powders can be recommended due to their close-meshed gel network and better whey drainage. MF concentrates have a high casein/whey protein ratio anyway, therefore, heating can be omitted regarding the rennet gelation properties. However, heating has a positive effect on the viable cell count of the bacteria in both samples after spray drying—independent of the casein/whey protein ratio. This makes heating to an appropriate tool for concentrate and consequently powder functionalization, which increases the numbers of viable bacterial cells. We could show that the cheese yield is enhanced when using powders made of heated UF concentrates or using powders of unheated MF concentrates. In summary, this process allowed to reduce the whey protein drainage by removal or integration without impairing the renneting properties, whereby the cheese yield increased.

The most significant contribution to this manuscript was made by the doctoral candidate. This comprised the conception and design of experiments based on preceded critical literature review as well as major conduction of data analysis, data interpretation, and discussion. In addition, writing and editing of the manuscript was done by the doctoral candidate. The co-authors contributed to the project outline, the execution of experiments, the discussion of results, and the revision of the manuscript.

Adapted original manuscript[7]

Cold-renneted milk powders for cheese production: Impact of casein/whey protein ratio and heat on the gelling behavior of reconstituted rennet gels and on the survival rate of integrated lactic acid bacteria[8]

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Abstract: The idea was to develop powders for fresh/hard cheese or quark production comprised of milk proteins in optimal composition and functional properties for manufacturing each of those cheese types. The aim was to avoid whey protein drainage by their prior removal or by their heat-induced structural integration in the curd. The pre-renneted powders already contain additives like starter cultures and calcium chloride to instantaneously form homogeneous curds upon reconstitution. The impact of the casein/whey protein ratio (86 : 14 by ultrafiltration and 98 : 2 by microfiltration) and upfront heat treatment (80 °C/30 min) on the gelling behavior of reconstituted rennet gels and on the survival rate of integrated *Lactobacillus paracasei* ssp. *paracasei* F19 was investigated. The assessment criteria for the rennet gelation were curd firming rate, gel strength, and whey drainage. Furthermore, the amount of integrated whey proteins and the resulting cheese yield were evaluated. It could be shown that heating had a positive effect on the viable cell count of the bacteria after spray drying and on the gelation behavior of the reconstituted ultrafiltration concentrates. The curd firming rate and the gel strength could be increased to higher values than the reconstituted microfiltration concentrate at 25% total solids.

Keywords: Cheese manufacture; Ultrafiltration; Microfiltration; Cold-renneting; Spray drying; Whey protein denaturation; milk protein concentrates; milk protein powders

3.4.1. Introduction

During cheese manufacture, 70% of the starting amount of milk is converted to sweet whey, which is commonly purified to obtain the isolated whey proteins. In some parts of the world with lacking infrastructure for whey processing, the whey must be discarded and then potentially creates environmental burden. A novel concept to avoid whey disposal studied in this work is powder-based cheese manufacture with high yield due to an increased casein/whey protein ratio or the integration of the whey proteins in the cheese matrix.

To avoid losses of valuable whey proteins, heat treatment could be applied up to a certain degree to denature and structurally integrate the whey proteins in the cheese curd (Hinrichs, 2001). However, heat treatment prior to renneting affects the rennetability of the casein micelles due to whey proteins blocking the casein

[7] Adaptions refer to formatting issues: e.g., numbering of sections, figures, tables, and equations; abbreviations, manufacturer specifications, axis labeling, figure captions, and style of citation. References are listed at the end of this thesis, combined with the references of the other publications, to avoid redundancies.

[8] Originally published in: Foods (2021), 10(7), 1606.

micelle surface. Steffl (1999) and Steffl et al. (1999) reported on an impaired rennet gelation, if β-lactoglobulin denaturation exceeded 60%. Then, the binding sites (the κ-casein) are partly occupied and not accessible for the rennet anymore. In contrast to Steffl (1999) and Steffl et al. (1999), Anema et al. (2007) found that the observed retarded rennet gel formation was irrespective of whether denatured whey proteins were associated with the casein micelles or self-aggregated in the serum phase. Thus, these authors assumed that these complexes inhibit further aggregation and therefore, retard the gelation.

However, these detrimental effects of present whey protein aggregates on rennet gelation can be circumvented by ultrafiltration (UF) concentrating or increasing the casein/whey protein ratio, i.e., by reducing the whey protein content by microfiltration (MF) prior to heat treatment. Schreiber (2000) and Schreiber and Hinrichs (2000) reported that at casein concentrations above 8% the gel strength of heated UF concentrates was higher than the gel strength of pasteurized skim milk, even if all whey proteins were denatured. The integration of denatured whey proteins into the cheese matrix seems therefore possible if the casein concentration is high enough. Bulca (2007) found that skim milk with casein/whey protein ratios of 3.4 : 0.01% or 6.4 : 0.65% heat-treated at 140 °C for 10 s gelled as fast as pasteurized skim milk and showed the same gel firmness.

Based on these reports the idea of this study was to develop powders with the optimal composition for wheyless manufacturing of rennet based cheese types. The powder for fresh/hard cheese should have a high casein and a low whey protein concentration. For this, the casein concentration was increased, and the whey proteins were removed at the same time by MF in diafiltration mode. Considering powder for quark production, it should have a high total protein concentration, whereby the whey proteins are denatured and bound to the casein micelles. This can be achieved by heating UF retentates. The main advantages are on the one hand, the increased cheese yield due to the higher protein concentration, and on the other hand, the omitted sweet whey collection and purification for the consumer. Furthermore, the powders already have the optimal composition making the prior alteration of protein concentration and composition of the vat milk needless. The aim was to develop pre-renneted milk protein concentrate powders containing starter cultures and calcium chloride, ready to form homogeneous gel matrices upon rehydration.

A conceptionally similar study was conducted by Würth et al. (2016), who developed a spray drying process to encapsulate probiotics in milk matrices with the classic rennet gelation process for cheese manufacture. Skim milk containing *Lactobacillus paracasei* ssp. *paracasei* F19 was cold-renneted at 4 °C prior spray drying, where aggregation of the para-casein micelles does not occur (Bansal et al., 2008, 2007). Such so-called `capsule precursor powder´ formed the final water-insoluble hydrogel capsules upon rehydration at temperatures above 16 °C.

The fact that milk matrices are suitable for integrating bacteria in a protective environment was also shown by Khem et al. (2015). They reported that whey protein isolates (WPI) and skim milk have a higher protective effect on *Lactobacillus plantarum* than carbohydrates such as lactose or trehalose during drying. The reason was the crust formation in the early drying stage. This creates a shell and thus, prevents the droplet from overheating at the later drying stages. Moreover, the calcium present in milk was postulated to increase the intrinsic heat resistance of the lactic acid bacteria. Desmond et al. (2002) reported that adding 0.3 M sodium chloride to a suspension of reconstituted skim milk (20% (w/v)) and *Lactobacillus paracasei* resulted in a high degree of cross-protection for the bacteria against heat stress during spray drying.

Khem et al. (2016) investigated the effect of denatured whey proteins on the survival of *Lactobacillus plantarum* spray-dried in WPI, lactose, or WPI/lactose mixtures. The significantly higher survival rates in WPI compared to lactose or WPI/lactose mixtures led them to assume that the whey proteins have a protective effect on the bacteria. These authors thought that the whey proteins unfold during spray drying at an outlet temperature of approximately 70 °C and that these interact hydrophobically with the hydrophobic bacteria causing aggregate formation. As a result, the microorganisms embed in the capsule are protected against inactivation during spray drying. Based on these results it would be obvious that heated WPI solutions may have a better protective effect on bacterial cells because higher degrees of whey protein denaturation could be achieved. Moreover, Picot and Lacroix (2004) encapsulated *Bifidobacterium breve* in heated WPI solutions (80 °C/30 min). They achieved survival rates of 26% (corresponding to 10^9 cfu mL^{-1}, cfu = colony forming units) when spray drying with an air inlet and outlet temperature of 160 °C and 80 °C, respectively.

Based on the studies mentioned above, it was hypothesized that 1) cold-renneted skim milk UF and MF powders result in a homogenous gel matrix upon rehydration at temperatures below 16 °C; 2) UF concentration allows to integrate a high amount of denatured whey proteins without impairing the renneting properties; 3) the survival rate in the heated concentrates should be higher than in the unheated ones due to the protective effect of the whey protein aggregates.

The impact of the casein/whey protein ratio (86 : 14 and 98 : 2) and heat treatment (80 °C/30 min) on the gelling behavior of reconstituted rennet gels (meaning the reconstituted, gelled concentrates) and on the survival rate of integrated *Lactobacillus paracasei* ssp. *paracasei* F19 was investigated. The assessment criteria for the rennet gelation were curd firming rate, gel strength, and whey drainage. In addition, the amount of integrated whey proteins and the resulting cheese yield were evaluated. Furthermore, the viable cell count was determined after spray drying and during powder storage at 4, 10, and 20 °C during 103 days of storage.

This study was designed to form the base for `instant´ cheese production based of pre-renneted powders containing a high number of viable cells and all required ingredients forming cheese, but without whey protein drainage. As a powder with a high storage stability, transport across long distances could be possible, while the lactobacilli would be dormant due to lacking water, but rapidly activated upon rehydration.

3.4.2. Materials and Methods

3.4.2.1. Cold-renneted milk protein powder production

In this study, four cold-renneted skim milk powders varying in casein/whey protein ratio and whey protein denaturation were produced. Figure 3.23 shows the related process scheme.

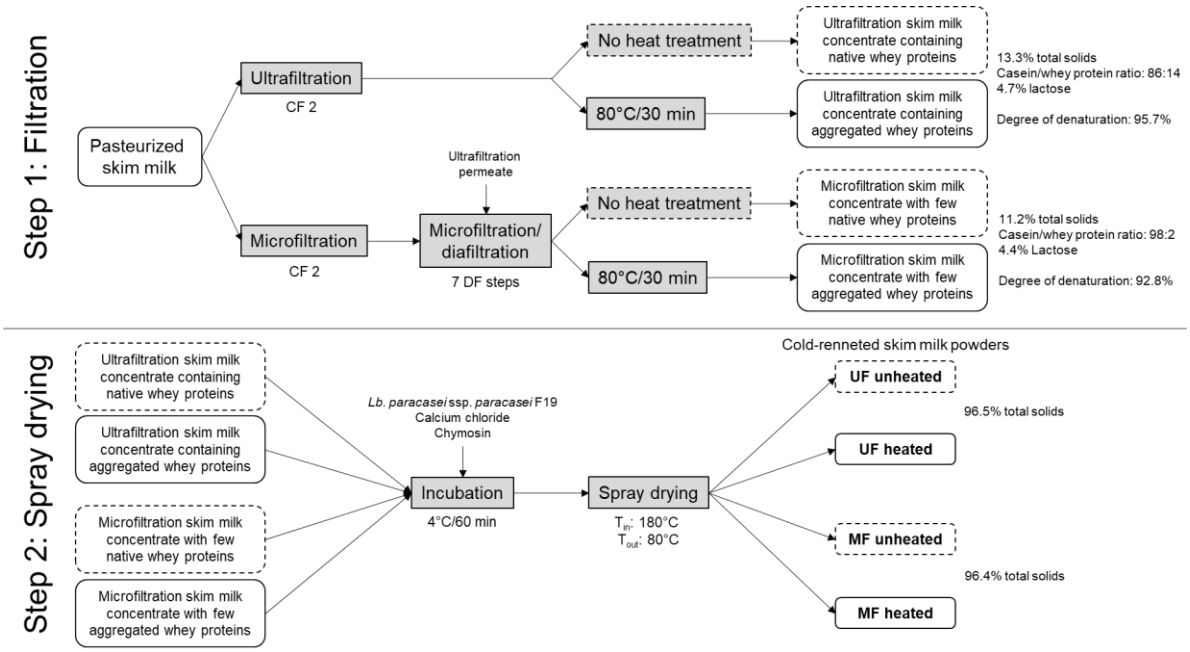

Figure 3.23 Processing scheme for cold-renneted skim milk powder production. CF: concentration factor; DF: diafiltration; UF: ultrafiltration; MF: microfiltration.

Pasteurized skim milk (74 °C/28 s, Molkerei Weihenstephan GmbH & Co. KG, Freising, Germany) was ultrafiltered (UF) with a polymeric spiral-wound membrane (10 kDa, DSS Silkeborg AS, Silkeborg, Denmark) and microfiltered (MF) with a ceramic module (0.14 µm, TAMI Industries, Nyons, France). During UF, the total protein concentration was increased from 3.8 to 6.9%. During MF and diafiltration, the casein concentration was increased from 3.3 to 5.5%, whereas the whey protein content decreased from 0.5 to 0.1%. UF permeate was used as diafiltration medium to keep the natural milk milieu. Hence, the lactose concentration was constant between 4.7 and 4.4% in the UF and MF concentrates, respectively. The filtration temperature was 50.0 ± 1.0 °C. Casein and whey protein content (according to Dumpler et al., 2017) and lactose content (according to Schmitz-Schug et al., 2013) were determined by reversed-phase high performance liquid chromatography (RP-HPLC).

Since the concept was to integrate the whey proteins in the cheese matrix by denaturation, each concentrate was split in two halves (see Figure 3.23). One half remained unheated, whereas the other half was heated at 80 °C for 30 min under steady stirring in a water bath to induce whey protein aggregation. The degree of whey protein denaturation was calculated according to Warncke et al. (2022). The final degrees of denaturation were 95.7 ± 0.7% for the UF concentrate and 92.8 ± 0.5% for the MF concentrate. All samples were stored at 4.0 ± 1.0 °C overnight.

Before spray drying, the probiotic strain *Lactobacillus paracasei* ssp. *paracasei* F19 (in the following *Lb. paracasei*) (Chr. Hansen A/S, Hørsholm, Denmark), calcium chloride (Effinger Klaus Käser- & Imkerbedarf, Sonthofen, Germany), and rennet (CHY-MAX® M 1000, Chr. Hansen A/S, Hørsholm, Denmark) with an enzyme activity of 1000 IMCU L^{-1} (international milk clotting units) were added to the four concentrates. The concentrations were 10.56 g culture (corresponds to $2.9x10^9$ cfu mL^{-1}) and 0.15 mL calcium chloride per kg protein concentrate. The chymosin was added with a concentration of 2.303 µL per gram casein. The concentrates were gently stirred for 10 min to distribute the ingredients homogeneously before resting for 50 min to give the chymosin time for hydrolysis of the κ-casein.

Spray drying was performed by a NIRO Atomizer (GEA Group, Düsseldorf, Germany). The inlet air temperature was 180.0 ± 1.9 °C and the outlet air temperature was 80.0 ± 2.2 °C to achieve a moisture content below 4% (w/w) for a good powder quality (Písecký, 2012) and powder storage stability (Ananta et al., 2005; Teanpaisan et al., 2012). The feed flow rate was 0.3 ± 0.03°L min^{-1} and the disk rotation speed was 15.000 rpm. The double-walled feed tank was tempered to 4.0 ± 0.5 °C to avoid a premature aggregation of the para-casein micelles.

The powders were stored in aluminum compound foil bags to avoid oxygen migration through the packaging material and to prevent the powders from UV radiation. The storage temperatures were 4, 10, and 20 °C to investigate the impact of the storage temperature on the viable cell count of the bacteria. The moisture content and the a_w-value of the powders were determined by the CEM Smart6 Turbo (CEM GmbH, Kamp-Lintfort, Germany) and the HygroLab C1 (Rotronic Messgeräte GmbH, Ettlingen, Germany), respectively. Each powder was produced twice.

3.4.2.2. Viable cell count of Lactobacillus paracasei ssp. *paracasei F19*

Before determining the viable cell count in the powders, the bacteria were isolated enzymatically according to Heidebach et al. (2010). For this, 1 g powder was mixed with 1 mL phosphate buffer (pH 7), 1 mL of the protease from *Lactobacillus amyloliquefaciens* ssp. *amyloliquefaciens* (Sigma-Aldrich, St. Louis, USA), and 7 mL deionized water (10 °C) in a 50 mL falcon tube. After 65 min in the New Brunswick™ Innova® 42/42R Incubator (Eppendorf AG, Hamburg, Germany) at 40 °C and shaking at 110 rpm, the powders were dissolved and the rennet gel, which built inevitably upon rehydration, was disintegrated.

The colony forming units (cfu) were determined using a serial dilution in Ringer's solution (Merck KGaA, Darmstadt, Germany) up to 10^{-7}, which was subsequently plated on MRS agar. The plates were incubated anaerobically at 37 °C for 48 h. Plates with up to 300 cfu were used for calculating the cfu per mL according to the established microbiological standard method (Goldman and Green, 2009). All serial dilutions were performed in duplicate.

The survival rate was calculated by Equation (3.13), where N_0 is the viable cell count (cfu mL^{-1}) after spray drying (corresponds to day 0) and N is the viable cell count after x days of storage.

$$Survival\ rate\ (\%) = \left(\frac{N}{N_0}\right) \cdot 100\% \qquad (3.13)$$

3.4.2.3. Surface hydrophobicity of Lactobacillus paracasei ssp. *paracasei F19 under heat stress*

The change in surface hydrophobicity of *Lb. paracasei* upon heating was tested according to Khem et al. (2016). For this, 15.0 ± 0.02 g of the *Lb. paracasei* culture was mixed with 50 mL of 10 °C deionized water in duplicate. To induce heat stress, one suspension was warmed to 45 °C under steady stirring in a water bath for 10 min according to Haddaji et al. (2015). Afterward, it was cooled on ice for 5 min.

25 mL of the unheated and heated suspension was mixed with 5 mL hexadecane in a falcon tube. After 15 min of incubation, the supernatant was clear (bacteria were hydrophilic) or milky white (bacteria were hydrophobic and solved in the hydrophobic solvent).

3.4.2.4. Curd firming rate and strength of reconstituted rennet gels

To evaluate the impact of the casein/whey protein ratio and denatured whey proteins on the rennet gelation properties of the cold-renneted reconstituted concentrates, the curd firming rate and the gel strength were determined.

Before the measurements, the powders were redispersed in 10 °C deionized water to 10 and 25% (w/w) total solids. To avoid a premature aggregation, the beaker was constantly on ice. After homogenizing with the Ultra-Turrax (IKA-Werke GmbH & CO. KG, Staufen, Germany) at 10,000 min^{-1} for 5 min, the samples were stored at 4 °C overnight under steady stirring. Before the measurements, the samples were homogenized again under the same conditions to ensure full powder solubilization (Warncke and Kulozik, 2021, 2020).

For both measurements, the MCR 302 rheometer (Anton Paar GmbH, Graz, Austria) equipped with the cone/plate geometry (d = 50 mm, 2°) was used. The sample volume was 1.15 mL. Oscillation at a constant deformation (0.01 %) and frequency (1 Hz) was applied for 7.2 min, whereby the sample formed a gel. At the same time, the temperature increased linearly from 4 to 40 °C. Having reached 40 °C, the temperature was held for 30 min. The curd firming rate was defined as the increase of the storage modulus G' within 300 s. The curd firming rate was calculated by Equation (3.14), where 0 s is that time, where the temperature reached 40 °C.

$$Curd\ firming\ rate\ (Pa\ s^{-1}) = \frac{G'_{300\ s} - G'_{0\ s}}{300\ s} \tag{3.14}$$

For measuring the gel strength, an amplitude sweep was applied. First, the samples were sheared at 500 s^{-1} for 1 min at 4 °C to give them time to equilibrate. After resting for 30 s, the temperature increased to 40 °C within 10 min to induce rennet gel formation. After reaching and holding 40.0 ± 0.02 °C for another 10 min, the oscillating amplitude sweep (logarithmic ramp from 0.01 to 100% deformation at a constant frequency of 1 Hz) was applied to determine the gel strength in the linear viscoelastic region (LVR) (corresponds to 0.01-0.05% deformation). All measurements were performed in duplicate.

3.4.2.5. Whey drainage, whey protein integration in the curd, and cheese yield

The whey drainage, which occurs upon curd cutting and pressing, is an important criterion in cheese manufacture which defines the dry matter and therefore, the hardness of the final cheese. For whey drainage determination, 40.0 ± 0.5 g of the reconstituted, homogenized sample was weighed in a 50 mL falcon tube. After 1 h of incubation at 40 °C, the samples were centrifuged at 4,000 x g for 45 min at 20 °C. The supernatant

was immediately weighed, and the whey drainage calculated by Equation (3.15), where m_{serum} (g) is the serum mass and m_0 (g) the mass of the whole sample.

$$Whey\ drainage\ (\%) = \frac{m_{serum}}{m_0} \cdot 100\% \quad (3.15)$$

To evaluate the impact of heat on the whey protein integration in the cheese matrix, the whey protein concentration in the sweet whey was analyzed by RP-HPLC. The cheese yield was calculated as follows (Equation (3.16)), where m_0 is the mass of the whole sample.

$$Cheese\ yield\ (kg\ 100\ kg^{-1}) = \frac{m_{curd}}{m_0} \cdot 100 \quad (3.16)$$

The highest cheese yield was expected for the heated UF sample, because the number of proteins, which can structurally contribute to the gel network, was highest. Both analyses were performed in duplicate.

3.4.2.6. Statistical analysis of data

Origin 2021 (OriginLab Corporation, Northampton, United States) was used to plot graphs and RStudio, Inc., 2019 (version 1.2.5033, Boston, MA, United States) was used for statistical analysis. Statistical significances ($P \leq 0.05$) were evaluated using one-way analysis of variance (ANOVA) combined with Tukey's HSD post-hoc test. Significant differences were calculated for values of different variables measured in two independent powders.

3.4.3. Results and Discussion

3.4.3.1. Survival of Lactobacillus paracasei ssp. *paracasei F19 after spray drying and during powder storage*

Figure 3.24 illustrates the viable cell count of the ultrafiltration (UF) and microfiltration (MF) samples before and after spray drying. Cell counts N_0 before spray drying of the unheated and heated UF and MF samples were not significantly different ($P = 1.00$) with ~3×10^9 cfu mL^{-1}. Spray drying caused a significant reduction of the viable cell count by ~2-2.5 log levels to 3.7×10^6 - 1.2×10^7 cfu mL^{-1} in all four samples ($P \leq 0.001$). This means a survival rate of 13 and 31% in the unheated UF and MF powders, respectively. This is in the range reported by Teanpaisan et al. (2012), who spray-dried *Lb. paracasei* in reconstituted skim milk with 20% total solids at 170/80 °C air inlet/outlet temperature.

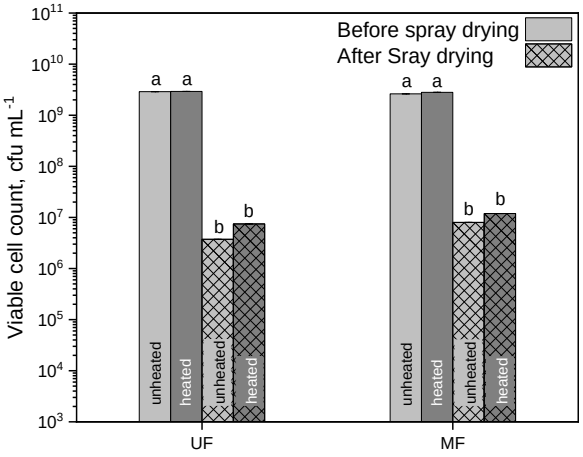

Figure 3.24 Viable cell count before and after spray drying of UF and MF. The Standard deviations of all samples before spray drying were ± 5x10^4 cfu mL^{-1} and after spray drying 2x10^3 cfu mL^{-1} (presented, but not visible due to scale). Letters indicate the statistical significance at $P \leq 0.05$.

The differences between the UF and MF concentrates can be attributed to the differences in total solid content of the concentrates before spray drying. Due to whey protein depletion, the total solid content in the MF concentrate was 11.2%, whereas the UF concentrate contained 13.3% total solids. The higher the total solid content, the faster a droplet reaches and exceeds the wet bulb temperature. Consequently, the heat stress for bacteria at a constant residence time is higher (Würth et al., 2018). However, the cell count was still above 3x10^6 cfu g^{-1}. The viable cell count after spray drying can be increased by inoculating a higher cell number, by decreasing the total solid content of the feed solution, or by decreasing the drying temperature (Würth et al., 2018). However, the moisture content needs to be considered as it should not exceed 4% (w/w) for a good powder quality (Písecký, 2012) and powder storage stability (Ananta et al., 2005; Teanpaisan et al., 2012).

As hypothesized, the heated samples contained more viable cells after spray drying than the unheated ones. The survival rate in the heated UF powder was 25%, whereas the survival rate in the heated MF powder was 43%. It could be assumed that the heated milk constituents have a protective effect caused by hydrophobic interactions on the bacteria as already shown by Khem et al. (2016).

The surface hydrophobicity of the milk proteins can be altered by heating or renneting. During denaturation, the whey proteins unfold and expose their hydrophobic regions. They can either bind to κ-casein on the casein micelles' surfaces, to serum κ-casein, or build disulfide-bonded and hydrophobically associated serum aggregates (Nair et al., 2013). These serum aggregates and casein/whey protein complexes bear a significantly higher surface hydrophobicity than unheated casein micelles (Guyomarc'h et al., 2007; Jean et al., 2006). Another way to turn the casein micelles hydrophobic is renneting: Rennet cleaves the hydrophilic κ-casein on the surface into para-κ-casein and casein macropeptide. Consequently, the casein micelles become more hydrophobic. This raised the question whether *Lb. paracasei* in our study turned hydrophobic during spray drying as well, which would confirm the hypothesis of Khem et al. (2016). The hydrophobicity test indicated the change from a hydrophilic to a hydrophobic surface of the bacterial cells when heat stressed, just as the bacteria experience it in the spray dryer. Due to the prior heating, more denatured—and hence, hydrophobic—whey proteins are available in the heated UF and MF samples, which potentially act as a protective shield for the

bacteria against overheating. Consequently, the viable cell count in the heated samples were higher after spray drying.

The survival rate of the lactobacilli cells during powder storage at different temperatures were further investigated. Figure 3.25 a shows the survival rate of *Lb. paracasei* during 103 days of storage at 4, 10, and 20 °C. Day 0 corresponds to the viable cell count after spray drying. The powder composition as well as the state (unheated/heated) had no significant impact on the survival rate during storage ($P = 0.86$). It turned out that the highest survival rates were achieved at 4 °C with 51% after 25 days and 45% after 103 days of storage. The survival rates of the cells in the powders stored at 10 °C showed a slightly lower survival rate with 46% after the first 60 days of storage. Storage at 20 °C yielded the lowest survival rate with a loss of almost 80% within the first 25 days. After 103 days, only 6.5% of *Lb. paracasei* were still vital, which confirms results reported in Ananta et al. (2005), Foerst et al. (2012), Gardiner et al. (2002), Teanpaisan et al. (2012), and Teixeira et al. (1996). The reason for this could be the damaged lipid cell membrane and the resulting lipid oxidation (Teixeira et al., 1996). The powders were simultaneously analyzed regarding their moisture content and a_w-value. Figure 3.25 b shows the change in moisture content and a_w-value over the powder storage period. It turned out that the moisture content increased only slightly from 3.6 to 4.1% and the a_w from 0.16 to 0.2 in all powders within the first 21 days of storage. The a_w was around 0.2, which was in the suitable range (max. 0.65) for bacterial survival in milk-based powders (Kosanke et al., 1992). This allows the losses in survival rate to be attributed to the temperature only, which seemed to be the main factor affecting the survival rate of the bacteria during powder storage. Nevertheless, even after 103 days of storage at 20 °C, the viable cell count was still above 10^6 cfu g^{-1}.

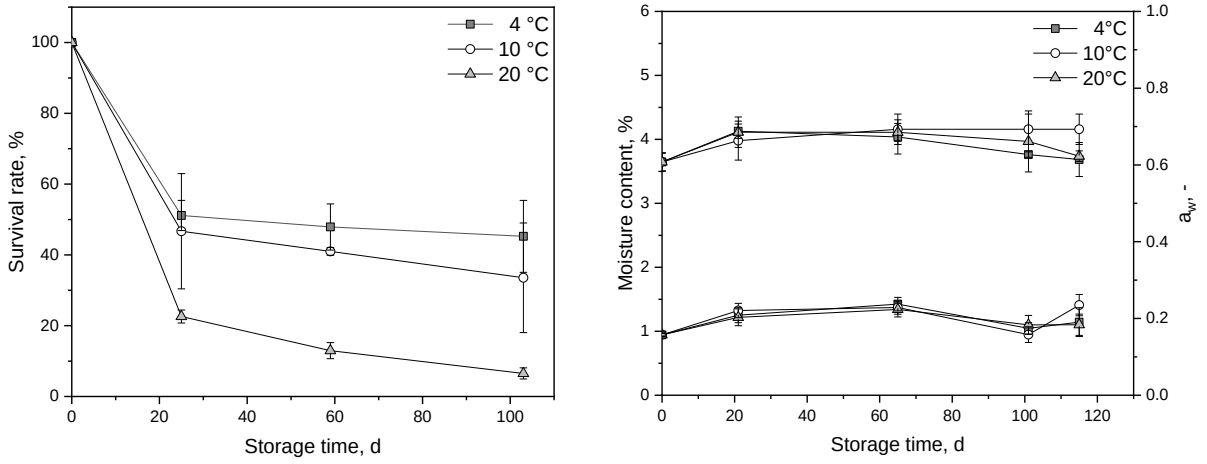

Figure 3.25 Survival rate of *Lactobacillus paracasei* ssp. *paracasei* F19 (a) and moisture content and a_w (b) as a function of storage time.

In summary, it can be said that the storage and transport of the powders at 20 °C is possible and that a viable cell count of 10^6 cfu g^{-1} can be ensured when inoculating the milk concentrates with $N_0 = 2.9 \times 10^9$ cfu mL^{-1}.

3.4.3.2. Rennet gelation behavior of reconstituted cold-renneted skim milk concentrates

In the following chapter, the curd firming rate, gel strength, whey drainage, whey protein integration, and cheese yield of the reconstituted cold-renneted skim milk concentrates are presented.

3.4.3.2.1. Curd firming rate

The curd firming rate is defined as the increase of the storage modulus G′ within 300 s. Figure 3.26 illustrates the curd firming rate of unheated and heated UF and MF rennet gels with 10 and 25% (w/w) total solids.

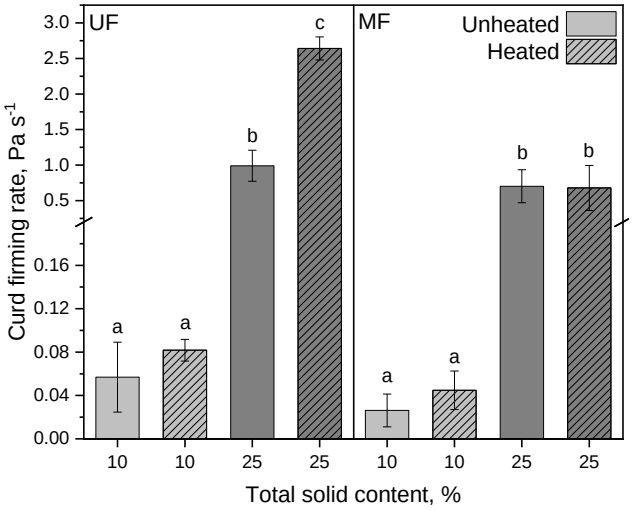

Figure 3.26 Curd firming rate of unheated and heated reconstituted UF and MF rennet gels with 10 and 25% total solid content. Letters indicate the statistical significance at $P \leq 0.05$.

Heated UF concentrate showed a higher curd firming rate compared to the unheated counterpart as it increased from 0.06 to 0.08 Pa s^{-1} at 10% total solids. At 25% total solids the curd firming rate significantly increased from 0.99 to 2.64 Pa s^{-1} ($P = 0.002$) (Figure 3.26). This indicates that the first phase of the renneting process, the enzymatic cleavage of κ-casein by hydrolysis at 4 °C prior spray drying, was not impaired by whey protein denaturation, and that aggregation occurred upon powder rehydration at elevated temperature. Anema et al. (2007) and Mollé et al. (2006) showed that chymosin is able to cleave the κ-casein in soluble heat-induced κ-casein/whey protein aggregates. However, our results are in contrast to those reported by Anema et al. (2007), where heating skim milk at 90 °C for 5-30 min resulted in a retarded rennet gelation process, and Steffl (1999) and Steffl et al. (1999), who reported that above a whey protein denaturation degree of 60%, milk remains liquid. Anema et al. (2007) observed a retarded gelation after heating (90 °C/15 min) as well and explained this with the disturbance of the denatured whey proteins in the aggregation process—independent of whether the whey proteins are complexed with the casein micelles or serum κ-casein. In this study, milk concentrates with either a higher total protein concentration or a higher casein/whey protein ratio compared to skim milk were used. Bulca et al. (2004), Waungana et al. (1998), Schreiber (2000), and Schreiber and Hinrichs (2000) already showed that concentrating as well as increasing the casein/whey protein ratio compensate these longer gelation times or weaker gels of ultra-high temperature-treated skim milk, so that rennet gelation is still possible even at high degrees of whey protein denaturation. It can be assumed that the increased hydrophobicity of the casein/whey protein complexes may not only protect the bacteria from cell death during spray

drying but could also be the reason for the higher curd firming rate in heated UF samples. This would be in accordance with the results found for the MF concentrates, where the unheated and heated samples behaved similarly, especially at 25% total solids. At 10%, the curd firming rate remained around 0.03 Pa s^{-1} and at 25% around 0.69 Pa s^{-1}. Here, the whey protein concentration was much lower, less casein/whey protein aggregates formed upon heating, and consequently, the hydrophobicity of the casein micelles was only altered by the rennet but not increased further by attached whey proteins.

3.4.3.2.2. Gel strength and whey drainage

The gel strength in the linear viscoelastic region (LVR) was determined by rheometry. This method provides insights regarding gel firmness without destructing the gel network like in penetration measurements. The gel strength displays the curd firmness after full formation before cutting. The same trends were observed for the gel strength in the LVR as for the curd firming rate. Figure 3.27 illustrates that a high curd firming rate went hand in hand with a high gel strength.

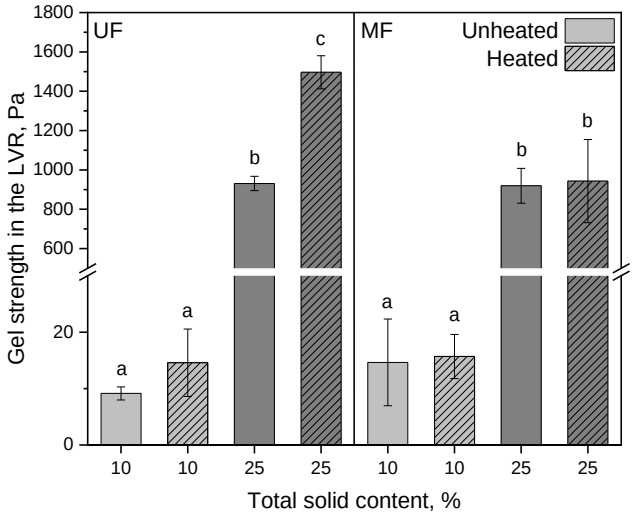

Figure 3.27 Gel strength in the linear viscoelastic region (LVR) of unheated and heated reconstituted UF and MF rennet gels with 10 and 25% total solid content. Letters indicate the statistical significance at $P \leq 0.05$.

The gel strengths of the UF and MF samples at 10% total solids were ~15 Pa, whereas the gel strength at 25% total solids were with 930 Pa significantly higher ($P \leq 0.001$) and increased up to 1,496 Pa when reconstituting heated UF concentrate powder. The higher gel strength at 25% total solids compared to 10% can be explained with the higher casein and colloidal calcium concentration (Figure 3.27). The more concentrated para-casein micelles are, the faster they contact each other and aggregate by calcium bridges. The strength of the network depends on the amount of involved casein micelles and hence, the amount of calcium bridges. The corresponding loss moduli for the unheated and heated UF samples at 10% total solids were 3.1 ± 0.4 Pa and 4.5 ± 1.8 Pa, respectively, and at 25% total solids they were 247.6 ± 15.3 Pa and 405.4 ± 32.9 Pa, respectively. The corresponding loss moduli for the unheated and heated MF samples at 10% total solids were 4.3 ± 2.1 Pa and 4.8 ± 1.0 Pa, respectively, and at 25% total solids they were 229.9 ± 15.3 Pa and 254.5 ± 63.2 Pa, respectively.

Our results demonstrate that a rennet gel made of heated, cold-renneted UF concentrate powder shows a significantly higher gel strength than the gel made from the unheated powder at 25% total solids as the gel strength increased from 931.1 to 1496.5 Pa ($P = 0.02$). Our results contrast with the results from literature showing softer and weaker gels made from heated skim milk (Anema et al., 2007; Singh and Waungana, 2001; Steffl, 1999). However, our results can hardly be directly compared to these studies by two reasons: first, we used UF and MF concentrates with a higher total protein concentration and/or a higher casein/whey protein ratio compared to skim milk; and second, in our process, hydrolysis and aggregation ran separately and not simultaneously like in the common renneting process. Our results also indicate that cold-renneting for 60 min at 4 °C allowed the rennet to hydrolyze enough κ-casein in the heated concentrates so that the subsequent aggregation upon rehydration was not impaired. Having separated hydrolysis and aggregation, our study allows to conclude that casein aggregation is not disturbed by denatured whey proteins. It can be assumed that a partly too fast aggregation—rather than the whey protein aggregates—hinder the rennet to hydrolyze all present κ-caseins leading to an incomplete hydrolysis and an insufficient aggregation in the common rennet gelation process. Singh and Waungana (2001) assumed that when heating UF concentrates, the nature of κ-casein/whey protein aggregates and the way in which casein micelles and whey proteins complex is different to that in heat-treated normal milk. These authors pointed out that the altered milk salt equilibrium in UF concentrates could also affect heat-induced changes in proteins and minerals.

The higher gel strength of the heated UF samples can be also explained by the higher serum holding capacity and consequently lower whey drainage. Figure 3.28 shows the whey drainage of the unheated and heated UF and MF samples at 10 and 25% total solids. As expected, a higher total solid content resulted in a lower whey drainage due to the increased solid/liquid ratio (78-86% at 10% total solids compared to 53 - 66% at 25% total solids). Furthermore, the heated UF samples showed a significantly lower whey drainage ($P \leq 0.001$) than the unheated ones. In contrast to that, the MF samples did not show any significant differences ($P = 0.99$) between heated and unheated at the same total solid content.

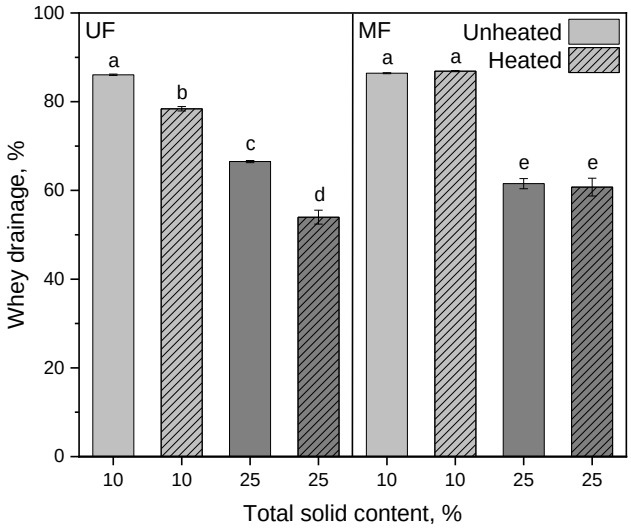

Figure 3.28 Whey drainage after incubation (t = 1 h, ϑ = 40 °C) and centrifugation at 4000 *x* g (t = 45 min, ϑ = 20 °C) of unheated and heated reconstituted UF and MF rennet gels with 10 and 25% total solid content. Letters indicate the statistical significance at $P \leq 0.05$.

After aggregation of the para-casein micelles, the gel network shrinks which intensifies the whey drainage. Casein micelles can be considered as round-shaped spheres allowing a close contraction of the network. On the contrary, irregularly shaped whey protein aggregates and casein/whey protein complexes of different sizes, which form upon heating, hinder the network sterically from a close contraction. As a result, more whey retains in the pores and the whey drainage decreases (Singh and Waungana, 2001).

3.4.3.2.3. Whey protein integration and cheese yield of reconstituted rennet gels

The whey protein integration and the cheese yield of heated reconstituted UF and MF rennet gels compared to their unheated counterparts were further investigated. Figure 3.29 a illustrates the whey protein concentration in the cheese matrix of unheated and heated UF and MF samples at 10 and 25% total solids. It turned out that heating allowed to integrate 81% of the whey proteins at 10% total solids and 87% at 25% total solids. In both cases this increase was significant ($P \leq 0.001$). Steffl (1999) also observed a 21% whey protein integration increase in heated cheese milk with 65% denatured whey proteins compared to unheated milk. On the contrary, only 4.7% and 9.1%, respectively, of the whey proteins are retained in the gel network without heating. This is in accordance with Warncke and Kulozik (2021) who observed a ~5% whey protein retention in the cheese matrix of renneted MC88- and MPC85-enriched skim milk as well. Since the whey protein concentration in the MF samples was lower, the total amount of whey proteins in the cheese matrix was lower. However, up to 80% of the available whey proteins could be integrated as well. Interestingly, already 63% of the whey proteins in the unheated MF samples at 10% total solids and 49% at 25% total solids were integrated in the matrix. Heating did not significantly increase the whey protein concentration in the cheese matrix ($P = 0.17$) of the MF samples at 10% total solids, but significantly at 25% ($P \leq 0.001$). Now the question was whether the heat treatment of MF retentates has an impact on the cheese yield (Figure 3.29 b).

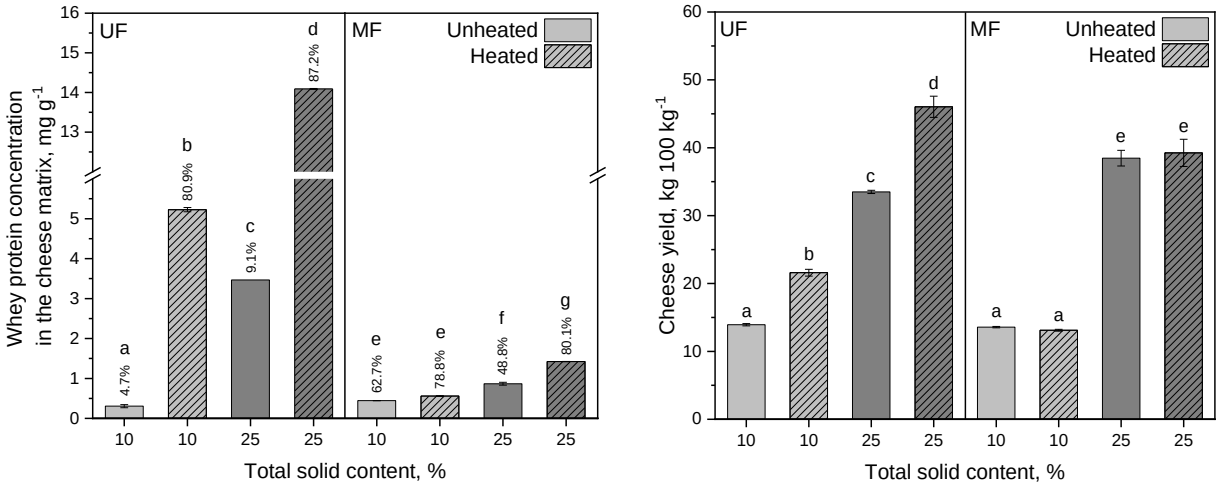

Figure 3.29 Whey protein concentration in the cheese matrix (a) and cheese yield (b) after incubation (t = 1 h, ϑ = 40 °C) and centrifugation at 4000 *x* g (t = 45 min, ϑ = 20 °C) of unheated and heated reconstituted UF and MF rennet gels with 10 and 25% total solid content. Letters indicate the statistical significance at $P \leq 0.05$.

It turned out that heating MF concentrates had no beneficial effect on the cheese yield, as it remained around 13 kg 100 kg^{-1} at 10% total solids and around 39 kg 100 kg^{-1} at 25% total solids. Independent of whether the MF concentrate was heat-treated before spray drying or not, the cheese yield as well as the rennet gelation properties remained the same ($P = 0.99$). In contrast to that, the cheese yield of the UF samples could be increased significantly by heat treatment ($P \leq 0.001$). At 10% total solids the cheese yield increased by 54% to 22 kg 100 kg^{-1} and at 25% by 37%. With 46 kg 100 kg^{-1} the heated UF sample with 25% total solids resulted in the highest cheese yield of all samples. The reason is a higher amount of integrated whey proteins as well as a higher serum retention in the pores (Guinee et al., 1998; Hinrichs, 2001). The unheated and heated MF samples (25% total solids) were slightly lower with a cheese yield of 39 kg 100 kg^{-1} but still higher than the unheated UF sample at the same total solid content. Steffl (1999) also reported on a 25% cheese yield increase of heated cheese milk with 65% whey protein denaturation. However, with increasing whey protein denaturation the yield increase decreased due to the soft curd texture resulting in a high amount of cheese dust, which gets lost during whey drainage (Steffl, 1999). This can be avoided when using heated concentrates since the gel strength was increased by heating as shown in Figure 3.27.

3.4.4. Conclusions

This study demonstrates that the casein/whey protein ratio and heat affect the gelation behavior of reconstituted rennet gels and the survival rate of integrated *Lactobacillus paracasei* ssp. *paracasei* F19. Ultrafiltration (UF) and microfiltration (MF) concentrates were cold-renneted and mixed with *Lb. paracasei* and calcium chloride before spray drying. Aggregation occurred upon powder rehydration resulting in gel matrices with 10 and 25% total solids.

Based on the data obtained, the hypotheses could be confirmed in that 1) cold-renneted skim milk UF and MF powders result in a homogenous gel matrix upon rehydration at temperatures below 16 °C, when they got homogenized; 2) concentrating allows to integrate up to 87.2% of the denatured whey protein without

impairing the renneting properties due to the sufficient amount of colloidal calcium and calcium bridges; 3) the survival rate in the heated concentrates was higher than in the unheated ones due to the more extensive whey protein denaturation and the probably more intense hydrophobic interactions.

The higher serum holding capacity of the UF curd makes this powder suitable for cheese types with higher moisture contents. For cheeses with a lower moisture content, the MF powders can be recommended due to their tightly meshed gel network and better whey drainage. MF concentrates have a high casein/whey protein ratio anyway, therefore, heating can be omitted regarding the rennet gelation properties. This process allowed to reduce the whey protein drainage by removal or integration without impairing the renneting properties, whereby the cheese yield can be increased. Heating is an appropriate tool for pre-treatment regarding powder functionalization, while increasing the number of viable integrated bacterial cells. This can be used for developing functional dairy foods containing active probiotics for example. If the amount of rehydration water could be chosen such that the final dry matter of a respective type of cheese would be reached, wheyless cheese manufacture could be performed anywhere, irrespective of local availability of cow´s milk.

Acknowledgments: The authors thank Günther Unterbuchberger for his assistance in performing the filtration and spray drying experiments. Moreover, we wish to express our gratitude to Claudia Hengst, Heidi Wohlschläger, and Hermine Roßgoderer for help in conducting the analytical measurements. Special thanks go to Michael Reitmaier and Roland Schopf for their support and valuable discussions.

4. Overall discussion

Intense and diverse research on milk powders' solubility has been conducted within the last decades. However, research is lacking regarding powders' functional aspects, although, they are routinely used for studies as substitute for fresh milk or milk protein concentrates. In this context, the review of full solubilization is often neglected. Therefore, the aim of this thesis was to investigate the impact of remaining powder particles on the flow behavior and rennet gelation properties of different milk protein powders. Further to that, a novel concept for powder-based instant cheese comprised of functionalized milk protein concentrate powders was developed. The main objective was to deplete or to integrate the whey proteins by heat denaturation for a high yield and a low whey protein drainage. Since an increasing concentration, an increasing casein/whey protein ratio, and heat aggregate formation impact the rheological properties inevitably, the viscosity of fresh heated concentrates obtained by micro- (MF) and ultrafiltration (UF) were investigated as well to better understand the impact of those three main and interaction-dependent effects. Caseins are known to be the main contributor to powders' poor dissolution behavior, to the viscosity, and to an impaired rennet gelation after heating. How to overcome these adverse effects during processing will be discussed in the following based on the obtained findings in section 3 of this thesis.

When powder is dissolved, the monomodal particle size distribution of skim milk should be targeted to obtain equivalent rheological and renneting properties (Warncke and Kulozik, 2021, 2020). The rehydration properties mainly depend on the composition, particularly in the presence or absence of fast dissolving components, such as lactose and whey proteins, and slow dissolving caseins (Mimouni et al., 2010). The compositions of all powders used in this thesis in the various chapters are given in Table 4.1. Besides the impact of high shear on the dissolution behavior, skim milk (SM), milk protein concentrate with 50 and 85% (w/w) protein (MPC50 and MPC85), and micellar casein powder (MC) containing 13.4% lactose were investigated regarding the hydration temperature. For functionality experiments (rennet gelation), micellar casein (MC88) and another MPC85 powder were used.

Table 4.1 Composition of the investigated dairy powders (mean ± standard deviation) (Warncke and Kulozik, 2021, 2020).

	Casein (%, w/w)	Whey protein (%, w/w)	Total protein (%, w/w)	Casein/ whey protein ratio	Degree of denaturation (%)	Lactose (%, w/w)	Minerals (%, w/w)	Total solids (%)
SM	34.2 ± 0.1	5.3 ± 0.3	39.5	87:13	49.1	46.4 ± 0.9	3.9 ± 0.0	95.1 ± 0.0
MPC50	45.1 ± 0.3	6.2 ± 0.0	51.3	88:12	34.8	33.1 ± 0.2	3.5 ± 0.0	94.1 ± 0.1
MPC85	63.8 ± 0.2	10.2 ± 0.0	74.0	86:14	47.0	4.0 ± 0.0	2.3 ± 0.0	91.7 ± 0.1
MC	65.6 ± 0.5	1.4 ± 0.2	67	98:2	60.8	13.4 ± 0.2	3.5 ± 0.0	95.9 ± 0.0
MC88	78.4 ± 0.5	5.6 ± 0.6	84	93:7	65.4 ± 0.8	1.4 ± 0.2	2.7 ± 0.0	94.4 ± 0.0
MPC85	70.5 ± 0.2	11.5 ± 0.1	82	86:14	42.7 ± 0.0	2.7 ± 0.0	2.4 ± 0.0	94.6 ± 0.0

The powders can be sorted according to their amounts of fast dissolving components and at the same time according to their solubility: MPC85 < MC < MPC50 < SM (Figure 3.1). Hydrophilic lactose acts as pathway for moisture transfer into the casein micelle during rehydration (Baldwin, 2010). A low lactose concentra-

tion goes hand in hand with a slow water penetration and hence, a bad dissolution behavior. Low temperatures like 4 °C and high temperatures like 50 °C seem to support the release of fast dissolving components and the dissolution of casein micelles in SM, MPC50, and partly MC (Warncke and Kulozik, 2020). Low temperatures are known to improve water absorption into the casein micelle and the release of calcium phosphate and β-casein, which leads to a partial loss in micellar integrity and, thus, to a more porous structure (Gaucheron, 2005). At higher temperatures such as 50 °C, the viscosity is decreased and the molecular mobility enhanced. These conditions allow the water to migrate faster into the powder particles, so that even slow dissolving casein micelles in SM and MPC50 fully rehydrate within 45 min under low shear stirring with a magnetic stir bar. However, MPC85 and MC do not fully dissolve (Figure 3.1). Higher shear treatments like in rotor/stator systems or high pressure homogenizers (HPH) were required.

In the dairy industry, two high shear units are widely implemented and would be suitable for powder redispersion: shear pumps like in powder mixers and HPH. In cheese manufacture, it is common to increase the vat milk with high protein powders like MPC85 or MC88 to increase the casein content, which in turn, results in a higher yield (Anema et al., 2006; Singh, 2007). Therefore, we enriched skim milk with MC88 and MPC85 to 4.5% casein concentration. Since the powders showed best rehydration properties in the shortest time at warm temperatures (Warncke and Kulozik, 2020), both mixtures were pre-sheared at 40 °C for 30 min under steady stirring before getting high shear treated in the rotor/stator system or HPH. The advantages of this process in the context of cheese manufacture are that the high shear treatment can run continuously, and the fully dissolved protein-enriched milk directly has the optimal temperature for renneting. The experiments were performed on a laboratory scale and on a pilot scale to assess their transferability. A colloid mill was used as rotor/stator system on a laboratory scale and a shear pump on a pilot scale. Furthermore, a laboratory and pilot HPH were utilized. The configurations for the desired shear rate of the stirred tanks, colloid mill, shear pump, and HPH are presented in Table 4.2.

Table 4.2. Configurations chosen for shear treatments in laboratory and pilot scale for the same shear rate calculated with Equations (3.2)-(3.5) (Warncke and Kulozik, 2021).

	Stirred tank	Colloid mill/ shear pump	Colloid mill/ shear pump	Colloid mill/ shear pump	Colloid mill/ shear pump	High-pressure homogenizer
Configuration laboratory scale	53 rpm	3170 min^{-1}	3487 min^{-1}	4026 min^{-1}	5088 min^{-1}	100 bar
Configuration pilot scale	33 rpm	1255 min^{-1}	1381 min^{-1}	1594 min^{-1}	2015 min^{-1}	100 bar
Calculated shear rate (s^{-1})	27	$3.3x10^3$	$3.6x10^4$	$4.1x10^4$	$5.2x10^4$	$6.0x10^7$

It turned out that on a laboratory scale as well as on a pilot scale, only HPH (100 bar, corresponds to $6.0x10^7$ s^{-1}) led to full powder solubilization. The flow conditions in the rotor/stator systems were insufficient—even at shear rates up to $7.4x10^4$ s^{-1}. For complete powder particle destruction (Figure 3.7); additional cavitation, which only occurs in HPH, was required (Figure 3.3, Figure 3.8). Then, the rheological behavior as well as the renneting properties remained unaffected by large disturbing powder particles, which increase the shear stress of MPC85 and MC dissolved in water on the one hand (Figure 3.2), and the gelation time (Figure 3.9), serum loss, and structure loss upon deformation (Figure 3.12) of MPC85- and MC88-enriched skim milk on the other hand. Further to that, large powder particles led to a decreased gel strength (Figure 3.10) and protein recovery in the sweet whey (Figure 3.11).

In the study of Warncke and Kulozik (2020) lactose seemed to be the deciding fast dissolving component regarding powder solubility, since the MC powder used in that study contained as much casein as the used MPC85, but 13.4% lactose compared to 4% in the MPC85. In contrast to that, in the study of Warncke and Kulozik (2021) the lactose concentrations of both powders were low with 1.4 and 2.7%, respectively. Although MC88 contained less fast dissolving components than MPC85, it showed a better rehydration in the shear range of $3.3x10^3$ to $4.1x10^4$ s^{-1}. Since the protein as well as the lactose concentrations in both powders were similar, we could conclude further that the casein/whey protein ratio, which defines the extent of protein interactions and aggregate formation, determines the powders' dissolution behavior.

In addition, protein interactions and whey protein denaturation occur as a result of the powder production process, which may be responsible for the powders' poor dissolution behavior. According to Oldfield and Singh (2005), the extent of protein interactions that occur upon pre-heating before evaporation and spray drying in milk powder production affects the powder solubility and shelf life. A high degree of whey protein denaturation and aggregation enhances the oxidative stability of whole milk powders and impairs their solubility. Oldfield et al. (2005) showed that evaporating skim milk up to 49% total solids and heat treating the concentrates at 64-74 °C did not significantly affect the whey protein denaturation. The authors explained this by the increased stability of the whey proteins at high total solid contents. We postulated that this could also be related to the high lactose concentration instead of the total solid content, and therefore, to the constantly high lactose/protein ratio during evaporation (Warncke and Kulozik, 2021). Whey proteins prefer the associated form in the presence of sugars to avoid unfavorable water-protein interactions (Arakawa and Timasheff, 1982; Timasheff, 2002). Consequently, lactose has a protective effect on β-lactoglobulin against denaturation if the lactose/protein ratio is high enough (Bernal and Jelen, 1985; Garrett et al., 1988; Jou and Harper, 1996; Plock et al., 1998; Spiegel, 1999).

However, milk protein concentrates for high protein powder production are usually evaporated and spray-dried directly after diafiltration without pre-heating (Singh, 2007). In diafiltered milk protein concentrates the lactose concentration is much lower. We observed no differences in the degree of whey protein denaturation of 0, 37, and 88% whey protein-depleted milk protein concentrates (diafiltered with ultrafiltration permeate) at the same total protein concentration after heating for 30 min at 80 °C (Warncke et al., 2022). This can be explained by the unaltered amount of reactive binding sites for the whey proteins (other whey proteins as well as the surfaces of casein micelles) in all three samples at the same total protein concentration. Therefore, the collision probability of the whey proteins with either casein micelles or other whey proteins was similar. Thus, differences in the degree of whey protein denaturation cannot be attributed to the casein/whey protein ratio alone (Warncke and Kulozik, 2021).

Furthermore, it could be observed that a high whey protein ratio led to an extensive whey protein aggregate growth in the serum phase (Warncke et al., 2022). The strong disulfide bonds between the whey proteins and the κ-casein and between the whey proteins themselves seem to be responsible for the poorer solubility of MPC85 compared to MC88 although the whey protein denaturation degrees in MPC85 and MC88 were 42.7 ± 0.0% and 65.4 ± 0.8%, respectively. The casein/whey protein ratio was considered as the more suitable measure for powder solubility evaluation compared to the degree of denaturation, since the number of formed aggregates are taken into account. A higher amount of whey proteins come along with more heat-induced aggregates (formed in the evaporator and spray dryer), which renders the powder less soluble (Warncke and Kulozik, 2021). The insoluble material in MPC85 predominantly consists of fused α_s- and β-caseins, forming a skin-like structure on the powder particles' surface, which inhibits the water penetration. Disulfide-linked κ-

casein/β-lactoglobulin complexes were present as well, but these play only a minor role in the formation of insoluble material (Havea, 2006; McKenna, 2000). Our results presented in Figure 3.8 indicate that whey protein aggregates as well as casein/whey protein complexes, which are more present in MPC85 than in MC88, are less soluble than casein aggregates, and are hence, the least dissolving components. Therefore, MPC85 required higher shear forces for aggregates' destruction and fully powder solubilization than MC88 (Warncke and Kulozik, 2021).

Upscaling experiments were performed in order to demonstrate that the results found on a laboratory scale are transferable to pilot scale. The target was to assess whether the particle sizes obtained on a laboratory scale are obtained on a pilot scale as well using a shear pump and a pilot HPH. Although the samples were homogenized at the same shear rates, the particle sizes in MC88- and MPC85-enriched skim milk treated on a pilot scale were larger compared to those on a laboratory scale. Only HPH at 100 bar led to the same particle sizes on both scales (Figure 3.13). The powder particles seemed to undergo less shear in the shear pump than in the colloid mill although the shear rates were calculated in the same way. Nevertheless, the rennet gelation behavior was in accordance with the laboratory results (Figure 3.14). Finally, the fitting equation (3.8) proved that the powder particles underwent less shear intensity in the shear pump. By means of the fit equation, the shear rates which needs to be applied on a pilot scale to obtain the same particle sizes as on a laboratory scale can be calculated. HPH can be taken over from laboratory to pilot scale unrestrictedly; the same pressures induce the same shear rates and hence, the same particle size distributions (Warncke and Kulozik, 2021).

The composition of milk protein concentrates is not only affecting the rehydration properties of the resulting powders, but also their viscosities before spray drying. Therefore, we investigated the viscosity of three milk protein concentrates of different casein/whey protein ratios (85 : 15, 92 : 8, 98 : 2) for total protein concentrations between 3.6% and 18.1%. For this, 0, 37, and 88% whey proteins were depleted by DF and the casein and whey protein fraction concentrated by UF, afterward. In the context of instant cheese powder production, the impact of whey protein denaturation on the rheological properties of the milk protein concentrates was assessed as well.

For all concentrates we observed that the viscosity exponentially increased with increasing total protein concentration. The difference between unheated and heated became larger with increasing whey protein concentration. The viscosity of the unheated 0% and 37% whey protein-depleted concentrates behaved similarly, whereas the viscosity of the 88%-depleted one noticeably differed from the others by increasing much stronger (Figure 3.18). The reasons are 1) the hydration and the net negative charge of the casein micelles; 2) the reduced free water, which increases the packing density of the caseins as the total protein concentration increases, although their volume fraction remains the same; 3) the distance between the micelles decreases, which in turn leads to more intense electrostatic repulsion. Therefore, the equally charged particles repel each other and force themselves in opposite flow directions. As a result, the flow resistance of the suspending medium increases (Ibanoğlu, 2002). At higher protein concentrations, the hairy layer of the casein micelles is compressed and interpenetrated, hence, their motions depend on hydrodynamic and inter-particle interactions (Nair et al., 2013). According to de Kruif (1998) and Horne (2003), the flow behavior of casein micelles dispersed in milk permeate at native pH can be described as for hard spheres. The viscosity can be expressed by the model of Krieger and Dougherty (1959), which takes the increasing particle interactions in concentrated suspensions into account.

The particle polydispersity in a concentrate increases with heat-induced protein complex formation (serum aggregates as well as casein/whey protein complexes). Either casein/whey protein complexes or soluble whey protein and κ-/α_s-casein/whey protein complexes form (Renhe and Corredig, 2018). The presence of casein/whey protein complexes and soluble whey protein aggregates increases the voluminosity and affect hence, the rheological properties of a concentrate (Anema et al., 2014; Langley and Temple, 1985; Snoeren et al., 1984b, 1982). The extent of complexation or aggregation depend on the protein concentration and the casein/whey protein ratio. Since the particle growth was greatest in the 0% whey protein-depleted concentrates after heating, they became more viscous compared to the unheated ones (Figure 3.18). The reason is that the free non-aggregated whey proteins enabled a closer packing of the protein volume fraction. Consequently, larger, less round-shaped aggregates formed upon heating, the packing density decreased, and the viscosity increased (Santamaría-Holek and Mendoza, 2010). The high whey protein ratio in the 0% whey protein-depleted samples resulted in a high whey protein concentration in the serum phase. This in turn facilitates collision between the molecules and whey protein aggregate growth. If the whey protein content was even if only partially reduced—like in the 37% and 88% whey protein-depleted concentrates—the whey protein aggregate growth is inhibited, because less reactive sulfhydryl-groups from β-lactoglobulin are available. Thus, less disulfide bonds (McKenzie et al., 1971; Smits and van Brouwershaven, 1980) also for the formation of casein/whey protein complexes are generated. Consequently, smaller aggregates form. This means that the casein micelles can be still considered as equally-sized spheres, which have a lower polydispersity and hence, particle packing density since the particle sizes of the heated 88% whey protein-depleted samples were almost not altered. In other words, with increasing polydispersity (88% < 0% < 37%), the packing density increases (Schaertl and Sillescu, 1994) and the viscosity at a given particle concentration decreases. However, the unheated and heated 88% whey protein-depleted concentrates showed high viscosities at high total protein concentrations as well due to the nature of native casein micelles (net negative charge and hydration).

To visualize the effects described above, the viscosities, dependent on casein/whey protein ratio, particle size, and polydispersity of the 0%, 37%, and 88% whey protein-depleted concentrates are schematically illustrated in Figure 4.1.

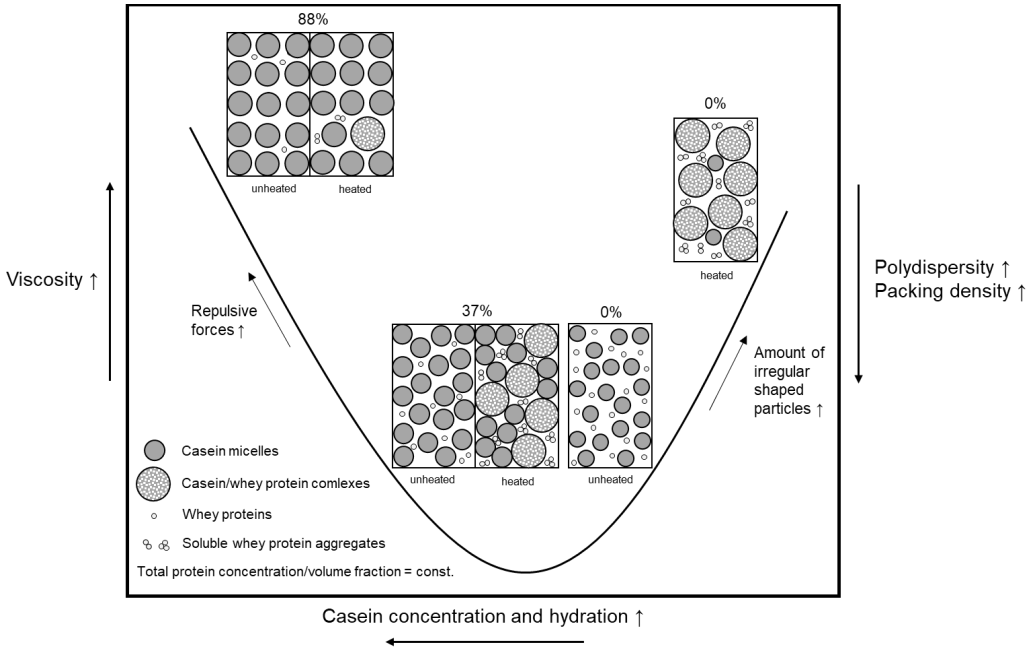

Figure 4.1 Model representation of the mechanisms underlying changes in the viscosity upon concentrating and heating at a constant total protein concentration/volume fraction of 0, 37, and 88% whey protein-depleted milk protein concentrates. Illustrations of the proteins are schematic and are not intended to meet the scale (Warncke et al., 2022).

Gaps between large particles (here, native casein micelles or casein/whey protein complexes) are filled by smaller ones (here, whey proteins or soluble aggregates) increasing the polydispersity and decreasing the viscosity. A high polydispersity is obtained at an increased casein/whey protein ratio and therefore, a lower amount of casein/whey protein complexes after heating, or a low casein/whey protein ratio without heating. Therefore, unheated and heated 37% and unheated 0% whey protein-depleted concentrates showed the lowest viscosities. The opposite is found when the 0% whey protein-depleted samples are heated: The large casein/whey protein complexes cannot be considered as round spheres anymore; irregular gaps may not be filled out perfectly by the whey protein aggregates resulting in a lower particle packing density and higher viscosity (Warncke et al., 2022).

By means of statistical modeling, we could show that the interactions between the total protein concentration and the whey protein ratio and between the total protein concentration and the degree of whey protein denaturation affect the viscosity most (Warncke et al., 2022). The apparent viscosity increases strongest with increasing total protein concentration and decreasing whey protein ratio (or increasing casein/whey protein ratio) (Figure 3.18, Figure 3.21). This is in accordance with Solanki and Rizvi (2001), who observed the same trends for MF retentates. 2- to 8-fold MF concentrates showed an increased shear stress with increasing total protein concentration and casein/whey protein ratio as well. The interaction of total protein concentration and degree of whey protein denaturation is in agreement with the results of Wolz et al. (2016), Kessler and Beyer (1991), and Pierre et al. (1977) who focused on whey protein concentrate solutions, casein/whey protein solutions with a ratio of 40/60, and UF concentrates, respectively. All authors observed an increased degree of whey protein denaturation with increasing total protein concentration.

All findings discussed before were utilized for the instant cheese powder production to avoid a too drastic viscosity increase of the feed concentrates—especially after cold-renneting—and to obtain a good powder solubility. In the study of Warncke et al. (2021), reconstituted rennet gels (meaning the reconstituted, gelled concentrates) made of unheated and heated (80 °C/30 min) UF and MF concentrates with casein/whey protein ratios of 86 : 14 and 98 : 2, respectively, were investigated regarding their gelling behavior and survival rate of integrated *Lactobacillus paracasei* ssp. *paracasei* F19.

The concentrate's total solid content before spray drying plays an important role for the survival rate of the lactic acid bacteria (Figure 3.24). Diafiltration led to 11.2% total solids in the MF concentrate, whereas UF resulted in 13.3%, hence, the survival rate was slightly higher in the MF samples. The higher the total solid content, the faster a droplet reaches and exceeds the wet bulb temperature. Consequently, the heat stress for bacteria at a constant residence time is higher (Würth et al., 2018). However, the cell count was still above $3x10^6$ cfu g^{-1} in the UF powder, although, it decreased by ~2.5 log levels after heating. Inoculating a higher cell number, decreasing the total solid content of the feed solution, or decreasing the drying temperature can increase the viable cell count after spray drying (Würth et al., 2018). However, the moisture content needs to be considered as it should not exceed 4% (w/w) for a good powder storage stability (Ananta et al., 2005; Teanpaisan et al., 2012) and powder quality (Písecký, 2012).

Since the survival rate in the heated UF powder was 25%, whereas the survival rate in the heated MF powder was 43%, we assumed that the heated milk constituents have a protective effect caused by hydrophobic interactions on the bacteria as already shown by Khem et al. (2016), who investigated the effect of denatured whey proteins on the survival of *Lactobacillus plantarum* spray-dried in WPI, lactose, or WPI/lactose mixtures. They thought that the whey proteins unfold during spray drying at an outlet temperature of approximately 70 °C and that these interact hydrophobically with the hydrophobic bacteria causing aggregate formation. A partial rather than a complete whey protein unfolding during spray drying is likely since we could show higher survival rates for prior heated concentrates than for unheated ones. The residence time in a spray dryer may be too short and the available water too less for full denaturation.

The surface hydrophobicity of the milk proteins alters upon heating or renneting. During denaturation, the whey proteins unfold and expose their hydrophobic regions. They can either bind to κ-casein on the casein micelles' surfaces, to serum κ-casein, or build disulfide-bonded and hydrophobically associated serum aggregates (Nair et al., 2013). These serum aggregates and casein/whey protein complexes bear a significantly higher surface hydrophobicity than unheated casein micelles (Guyomarc'h et al., 2007; Jean et al., 2006). Rennet cleaves the hydrophilic κ-casein on the casein micelle's surface into para-κ-casein and casein macropeptide. Consequently, the casein micelle becomes more hydrophobic. We found by means of a hydrophobicity test that the surface of the bacterial cells changed from hydrophilic to hydrophobic under heat stress, just as the bacteria experience it in the spray dryer. Due to the prior heating, more denatured, hydrophobic whey proteins are present in the heated UF and MF samples, which potentially act as a protective shield for the bacteria against overheating. Consequently, the viable cell count in the heated samples were higher after spray drying (Warncke et al., 2021).

It turned out that the highest survival rates during powder storage were achieved at 4 °C storage temperature with 45% after 103 days (Figure 3.25). Storage at 20 °C yielded the lowest survival rate; only 6.5% of *Lb. paracasei* was still vital after 103 days, which confirms the results reported by Ananta et al. (2005), Foerst et al. (2012), Gardiner et al. (2002), Teanpaisan et al. (2012), and Teixeira et al. (1996). The reason for this could be the damaged lipid cell membrane and the resulting lipid oxidation (Teixeira et al., 1996). Nevertheless, the storage and transport of the powders at 20 °C is possible and a viable cell count of 10^6 cfu g^{-1} can be ensured when inoculating the milk concentrates with $N_0 = 2.9 \times 10^9$ cfu mL^{-1} (Warncke et al., 2021).

The increased hydrophobicity of the casein/whey protein complexes possibly may not only protect the bacteria from cell death during spray drying but could also be the reason for the higher curd firming rate of the heated UF samples compared to the unheated counterpart (Figure 3.26). This indicates that the whey protein denaturation did not impair the first phase of the renneting process (the hydrolytic enzymatic cleavage of κ-casein at 4 °C prior spray drying) and that aggregation occurred upon powder rehydration at elevated temperature. Our findings contrast with other studies e.g., by Anema et al. (2007), Singh and Waungana (2001)and Steffl (1999), who reported on softer and weaker gels made from heated skim milk. However, our results can hardly be directly compared to these studies by two reasons: 1) we used UF and MF concentrates with a higher total protein concentration and/or a higher casein/whey protein ratio compared to skim milk; and 2) in our process, hydrolysis and aggregation ran separately and not simultaneously like in the common renneting process. Therefore, samples in our study neither resulted in a retarded nor in an inhibited rennet gelation. It is already known that concentrating as well as increasing the casein/whey protein ratio compensate these longer gelation times or weaker gels of ultra-high temperature-treated skim milk, so that rennet gelation is still possible even at high degrees of whey protein denaturation (Bulca et al., 2004; Schreiber, 2000; Schreiber and

Hinrichs, 2000; Waungana et al., 1998). Since hydrolysis and aggregation ran separately, it could be concluded that casein aggregation is not disturbed by denatured whey proteins. It can be assumed that a partly too fast aggregation—rather than the whey protein aggregates—hinder the rennet to hydrolyze all present κ-caseins leading to an incomplete hydrolysis and an insufficient aggregation in the common rennet gelation process (Warncke et al., 2021).

With the process described by Warncke et al. (2021) it was possible to integrate up to 87% of the whey proteins in the cheese matrix by heat treatment resulting in the highest possible cheese yield with 46 kg 100 kg^{-1}. A higher amount of integrated whey proteins goes hand in hand with a higher serum retention in the pores, which in turn, increases the yield (Guinee et al., 1998; Hinrichs, 2001). Steffl (1999) also observed a 21% whey protein integration increase and 25% cheese yield increase in heated cheese milk (65% whey protein denaturation) compared to unheated milk. However, as the whey protein denaturation increased, the yield increase decreased due to the soft curd texture resulting in a high amount of cheese dust, which gets lost with the drained whey (Steffl, 1999). Using heated concentrates avoids this, since the gel strength was increased by heating as shown in Figure 3.27.

Only a maximum of 9.1% of the whey proteins remain in the gel network without heating. This is in accordance with Warncke and Kulozik (2021), where we observed a ~5% whey protein retention in the cheese matrix of renneted MC88- and MPC85-enriched skim milk as well. Although, heating MF concentrates had a beneficial effect on the survival rate of integrated bacteria, it had no beneficial effect on the cheese yield since their whey protein concentration was low anyway. Independent of whether the MF concentrate was heat-treated before spray drying or not, the cheese yield as well as the rennet gelation properties remained the same (Warncke et al., 2021).

5. Conclusion

In this thesis, we investigated the impact of remaining powder particles on the flow behavior and rennet gelation properties of different milk protein powders. Further to that, a novel concept for powder-based instant cheese comprised of functionalized milk protein concentrate powders was developed. In this context, the viscosity of fresh, i.e. not dried, heated concentrates obtained by micro- (MF) and ultrafiltration (UF) were investigated as well, to better understand the impact of the factors total protein concentration, casein/whey protein ratio, heat, and of their interaction-dependent effects.

Applying high pressure homogenization (HPH) on poorly soluble dairy powder suspensions—especially those with a low content of fast dissolving components—appears to be indispensable for full powder dissolution. The flow conditions under low shear and high shear in rotor/stator systems were insufficient—even at shear rates up to 7.4×10^4 s^{-1}—for complete powder particle destruction of milk protein concentrate with 85% protein (w/w) (MPC85) and micellar casein powder (MC); additional cavitation, which occurs in HPH, was required. However, low protein powders like skim milk (SM) or milk protein concentrate powder containing 50% (w/w) protein (MPC50) dissolve completely under low shear at 4 °C overnight or at 50 °C within 45 min.

On the one hand, remaining powder aggregates increase the viscosity of reconstituted skim milk concentrates compared to freshly produced ones with the same composition. On the other hand, remaining powder particles impair the rennet gelation behavior due to the inaccessible proteins in the powder aggregates as well as the gel properties, which could be disturbed by large powder particles. An insufficient powder rehydration prolongs the rennet gelation time. The consequence could be a too early curd cutting in the cheese making process, leading to fluctuations in the resulting cheese properties and quality. MPC85-enriched skim milk showed softer, weaker gels and consequently, a reduced syneresis/serum loss, although the gelation times of MC88 and MPC85 were similar. This could be favorable for producing cheeses with high moisture contents. Moreover, remaining powder particles lead to a decreased whey protein concentration in the sweet whey. This can reduce the protein yield upon sweet whey purification.

HPH represents a (cost-)effective option for powder redispersion since it is already commonly established in dairies for milk homogenization. An alternative to the discontinuous pre-mixing in the stirred tank prior HPH could be the upstream installation of a powder mixer, where powder and solvent are continuously predispersed. This would reduce production time and costs further.

The cold-renneted instant cheese powders made of heated MF and UF concentrates resulted in homogenous gel matrices upon rehydration at temperatures below 16 °C, when they were homogenized. It could be shown that concentrating allows to integrate up to 87.2% of the denatured whey protein without impairing the renneting properties due to the sufficient amount of colloidal calcium and calcium bridges. In addition, more extensive whey protein denaturation and the probably more intense hydrophobic interactions led to higher survival rates in the heated concentrates than in the unheated ones. Moreover, the UF curd showed a higher serum holding capacity making this powder suitable for cheese types with higher moisture contents. For cheeses with a lower moisture content, the MF powders can be recommended due to their tightly meshed gel network and better whey drainage. Heating MF concentrates can be omitted regarding the rennet gelation properties since they have a high casein/whey protein ratio anyway.

This process allowed simultaneously the whey protein drainage reduction and the cheese yield increase by their removal or integration without impairing the renneting properties. Heating is an appropriate tool for

pre-treatment regarding powder functionalization with the positive side effect of higher survival rates of integrated bacterial cells.

Cold-renneting increases the viscosity. Therefore, the protein concentrate's viscosity prior cold-renneting and spray drying must not be disregarded. Considering casein micelle/whey protein interactions and the geometrical arrangement of the proteins and heat-induced protein complexes differing in size, the viscosity can be adapted. As the whey protein content is reduced and the casein concentration increased, repulsive forces become more dominant, due to the casein micelles' high net negative charge, particularly at high total protein concentrations. The polydispersity can be altered by varying the casein/whey protein ratio or by heating so that casein/whey protein complexes and soluble whey protein aggregates of different sizes form. A high polydispersity implies a high particle packing density and reduced viscosity. A whey protein depletion of 37% was sufficient to keep the repulsive forces between the casein micelles low enough, and to achieve a high polydispersity and particle packing density (even at 95% whey protein denaturation) so that the viscosity remained unaffected.

We concluded that both heating and concentrating are not limiting the viscosity inevitably. A partial whey protein depletion results in fluent and easy processible concentrates after heating—even at high total protein concentrations. The maximum total protein concentration should not exceed 9% if a high casein concentration is desired, to keep the casein micelles' hydration and their repulsive forces on an acceptable level at which the viscosity is still low and unaffected.

The instant cheese process can be used to develop functional dairy foods containing alive probiotics for example. By means of the statistical model the apparent viscosity of the (heated) feed concentrates prior spray drying can be calculated, which helps designing processes, such as the instant cheese process, where the apparent viscosity is a limiting factor.

If the powder is directly dispersed in the amount of water according to the desired dry matter of the cheese curd, wheyless cheese manufacture could be performed anywhere, irrespective of local availability of cow's milk.

6. Summary/Zusammenfassung

6.1. Summary

Investigating the solubility and rheology of milk protein powders is of great interest because they are commonly used in research or industrial applications as substitute for fresh milk concentrates. Highly concentrated protein powders are particularly challenging in terms of their solubility. Milk protein concentrate (MPC) and micellar casein (MC) powders are commonly used to increase the protein concentration of cheese milk. The focus in this work was on high shear treatments such as rotor/stator mixing systems and high pressure homogenization and its implications on the rheological and functional properties in terms of rennet gelation. The powders were either dissolved in water to compare their rheological profile to fresh concentrates or in milk—as this is routinely applied in cheese manufacture to increase the protein content—to evaluate the impact of remaining powder particles on the rennet gelation behavior.

Further to that, a concept for powder-based cheese manufacture was developed. The idea was to produce powders of unheated and heated cold-renneted skim milk concentrates, which form a gel upon reconstitution. The whey protein integration in the cheese matrix avoids the whey protein drainage resulting in a high yield. Since heating has a detrimental effect on the renneting properties of the casein micelles, the casein/whey protein ratio was varied to overcome this effect.

However, concentration inevitably affects the concentrate's viscosity—particularly after heat treatment. Another research question was therefore, which macroscopic mechanisms are responsible for changes in viscosity upon increasing the total protein concentration, varying the casein/whey protein ratio, and heating.

Skim milk powder (SM), milk protein concentrate powder containing 50% (w/w) protein (MPC50), milk protein concentrate powder containing 85% (w/w) protein (MPC85), and micellar casein powder (MC) were investigated regarding their rheological properties after dissolution at low shear (4, 20, and 50 °C) and high shear, namely high pressure homogenization (HPH) (50 °C, 500 bar). As assessment criteria, the rheological properties and particle sizes of reconstituted and fresh concentrates with same composition were compared. It turned out that the rehydration time is temperature-dependent. Similar particle size distributions were obtained after stirring at 4 °C overnight and 50 °C after 45 min. Samples stirred at 20 °C overnight still contained powder particles of 10 µm and larger. Therefore, this temperature is not a good choice for powder rehydration—even for powders with a good solubility such as SM—due to the poor reproducibility.

The powder aggregate's size is composition-dependent. A high casein/lactose ratio decreases the powder dissolving ability leading to bigger particles, higher shear stresses and stronger shear-thinning. Depending on the powder type, the particle sizes and shear stresses increased as follows: SM < MPC50 < MC < MPC85. SM and MPC50 were already fully rehydrated after 45 min at 50 °C. On the contrary, high protein powders like MPC85 and MC required an HPH step at 500 bar to disintegrate all powder aggregates. Doing so, the shear stress of MPC85 and MC drastically decreased.

After full rehydration of the powders, particle sizes and rheology of the reconstituted and fresh concentrates were the same. We concluded that the particle size is the most important factor of impact in terms of the rheological properties. The results of this thesis show that if the rehydration conditions are adapted to the powder type, fresh concentrates are completely substitutable with reconstituted concentrates regarding their rheology.

Since powder aggregates affect the rheological properties of reconstituted concentrates, their impact on the protein functionality was also evaluated as well. For this, the rennet gelation behavior of MC88- and MPC85-enriched skim milk was investigated. As commonly performed in cheese manufacture, skim milk was enriched with either MC88 or MPC85 to a casein concentration of 4.5% (w/w) for the experiments. The assessment criteria were particle size as a function of shear rate and the rennet gelation properties, namely, gelling time, gel strength, structure loss upon deformation, and serum loss. Furthermore, the casein, whey protein, and casein macropeptide (CMP) recovery in the sweet whey was determined to evaluate the shear- and hence, particle size-dependent protein accessibility. Since two high shear units are widely implemented in the dairy industry, these can be used for powder redispersion: shear pumps like in powder mixers and HPH. Therefore, we performed experiments on a laboratory scale first, using a colloid mill as rotor/stator system and a laboratory scale HPH. For the subsequent upscaling experiments a shear pump and a pilot scale HPH were used.

It turned out that the flow conditions in the rotor/stator systems were insufficient—even at shear rates up to 7.4×10^4 s^{-1}—for complete powder particle destruction; additional cavitation, as it occurs in HPH, was required for complete powder dissolution. An insufficient powder rehydration prolongs the rennet gelation time. The gelation time of MC88- and MPC85-enriched skim milk was similar; however, the addition of MPC85 resulted in softer, weaker gels and consequently, in a reduced syneresis/serum loss. Moreover, remaining powder particles decrease the whey protein concentration in the sweet whey.

During cheese manufacture, 70% of the amount of milk is converted into sweet whey, which is commonly purified to obtain the isolated whey proteins. However, in some parts of the world with lacking infrastructure for whey processing, the whey must be discarded and then potentially creates environmental burden. A novel concept to avoid whey disposal studied in this work is powder-based cheese manufacture with high yield due to an increased casein/whey protein ratio or the integration of the whey proteins in the cheese matrix.

The impact of the casein/whey protein ratio (86 : 14 and 98 : 2) and heat treatment (80 °C/30 min) on the gelling behavior of reconstituted rennet gels (meaning the reconstituted, gelled concentrates) and on the survival rate of integrated *Lactobacillus paracasei* ssp. *paracasei* F19 was investigated. The assessment criteria for the rennet gelation were curd firming rate, gel strength, and whey drainage. In addition, the amount of integrated whey proteins and the resulting cheese yield were evaluated. Furthermore, the viable cell count was determined after spray drying and during powder storage at 4, 10, and 20 °C during 103 days.

The total protein concentration was increased via ultrafiltration (UF) and the casein/whey protein ratio was adjusted via microfiltration (MF) in diafiltration (DF) mode. Since concentration increases the viscosity, especially after heat treatment, the mechanisms being responsible for changes in viscosity upon increasing the total protein concentration (3-18%), varying the casein/whey protein ratio (85 : 15, 92 : 8, 98 : 2), and heating (80 °C/30 min) were also investigated. The results indicate that both heating and concentration are not limiting the viscosity inevitably. A heated concentrate remains fluent and easy to process when whey proteins were partially depleted (here, 37%)—even at high total protein concentrations. If a high casein concentration is desired, the maximum total protein concentration should not exceed 9% to keep the casein micelles' hydration and their repulsive forces on an acceptable level at which the viscosity is still low and unaffected. By means of a statistical model, the apparent viscosity of milk protein concentrates of different total protein concentrations and casein/whey protein ratios can be calculated and better estimated, how pronounced the differences in viscosity might be after heating. This should help designing the instant cheese process, where the apparent viscosity is a limiting factor—especially after cold-renneting.

Therefore, the total protein concentrations were kept on an acceptable level during instant cheese powder manufacture to avoid a too drastic viscosity increase. Regarding the renneting properties, it turned out that heating had a positive effect on the gelation behavior of the reconstituted UF powder, since the curd firming rate as well as the gel strength increased to higher values than the MF samples at the same total solid content. The higher serum holding capacity of the curd makes this powder suitable for cheese types with higher moisture contents. For cheeses with a lower moisture content, the MF powders can be recommended due to their close-meshed gel network and better whey drainage. Since MF concentrates have a high casein/whey protein ratio anyway, heating can be omitted in terms of the rennet gelation properties. However, heating has a positive effect on the viable cell count of the bacteria in both samples after spray drying—independent of the casein/whey protein ratio. This makes heating to an appropriate tool for concentrate and consequently powder functionalization, which increases the numbers of viable bacterial cells. We could show that the cheese yield increases when using powders made of heated UF concentrates or using powders of unheated MF concentrates.

All aspects considered together, the hypotheses of this work were confirmed. Remaining powder aggregates increase the viscosity of reconstituted skim milk concentrates compared to freshly produced, equally compounded ones. Furthermore, they impair the rennet gelation behavior of protein-enriched skim milk due to the inaccessible proteins in the powder aggregates as well as the gel properties, which could be disturbed by large powder particles.

For the viscosity estimation of heated freshly produced milk protein concentrates, the casein micelle/whey protein interactions and the geometrical arrangement of the proteins and protein complexes differing in size must be taken into account. Cold-renneted UF and MF powders result in a homogenous gel matrix upon rehydration at temperatures below 16 °C, when they got homogenized. Concentrating allows to integrate up to 87.2% of the denatured whey protein without impairing the renneting properties due to the sufficient amount of colloidal calcium and calcium bridges. Furthermore, the survival rate in the heated concentrates was higher than in the unheated ones due to the more extensive whey protein denaturation and the probably more intense hydrophobic interactions between the bacteria and the denatured whey proteins.

6.2. Zusammenfassung

Die Untersuchung der Löslichkeit von Milchproteinpulvern und der Rheologie der Dispersionen ist von großem Interesse, da sie in der Forschung oder bei industriellen Anwendungen häufig als Ersatz für frische Milchkonzentrate verwendet werden. Hochkonzentrierte Proteinpulver stellen hinsichtlich ihrer Löslichkeit jedoch eine besondere Herausforderung dar. Milchproteinkonzentrat- (MPC) und micellare Caseinpulver (MC) werden häufig verwendet, um die Proteinkonzentration von Käsereimilch zu erhöhen. Der Schwerpunkt dieser Arbeit lag auf Behandlungen mit hohen Scherkräften wie in Rotor/Stator-Mischsystemen und Hochdruckhomogenisatoren und deren Auswirkungen auf die rheologischen und funktionellen Eigenschaften im Hinblick auf die Labgelbildung. Die Pulver wurden entweder in Wasser redispergiert, um ihr rheologisches Profil mit dem frischer Konzentrate zu vergleichen, oder in Milch – wie sie bei der Käseherstellung routinemäßig zur Erhöhung des Proteingehalts verwendet wird – um die Auswirkungen der verbleibenden Pulverpartikel auf das Labgelierverhalten zu bewerten.

Darüber hinaus haben wir ein Konzept für die Käseherstellung auf Pulverbasis entwickelt. Die Idee war, Pulver aus unerhitzten und erhitzten, kaltverlabten Magermilchkonzentraten herzustellen, die bei der Rekonstitution ein Gel bilden. Durch die Einbindung der Molkenproteine in die Käsematrix wird deren Drainage vermieden, was die Käseausbeute steigert. Da sich das Erhitzen nachteilig auf die Einlabungseigenschaften der Caseinmicellen auswirkt, wurde das Casein/Molkenprotein-Verhältnis variiert, um diesen Effekt zu kompensieren. Konzentrierung wirkt sich jedoch unweigerlich auf die Viskosität des Konzentrats aus – insbesondere nach der Hitzebehandlung. Eine weitere Forschungsfrage war daher, welche makroskopischen Mechanismen für die Veränderung der Viskosität bei Erhöhung der Gesamtproteinkonzentration, Variation des Casein/Molkenprotein-Verhältnisses und Erhitzung verantwortlich sind.

Magermilchpulver (SM), Milchproteinkonzentratpulver mit einem Proteingehalt von 50 % (w/w) (MPC50) und 85 % (w/w) (MPC85) sowie micellares Caseinpulver (MC) wurden auf ihre rheologischen Eigenschaften nach der Redispergierung bei niedriger Scherung (4, 20 und 50 °C) und hoher Scherung, d. h. Hochdruckhomogenisierung (HPH) (50 °C, 500 bar), untersucht. Als Bewertungskriterien wurden die rheologischen Eigenschaften und die Partikelgrößen von rekonstituierten und frischen Konzentraten derselben Zusammensetzung verglichen. Es zeigte sich, dass die Rehydratationszeit temperaturabhängig ist. Ähnliche Partikelgrößenverteilungen wurden nach Rühren bei 4 °C über Nacht und bei 50 °C nach 45 min erzielt. Proben, die über Nacht bei 20 °C gerührt wurden, enthielten noch Pulverpartikel von 10 µm und mehr. Daher ist diese Temperatur aufgrund der schlechten Reproduzierbarkeit keine gute Wahl für die Rehydrierung von Pulvern – selbst bei Pulvern mit guter Löslichkeit wie SM.

Die Größe des Pulveraggregats ist abhängig von der Zusammensetzung des Pulvers. Ein hohes Casein/Lactose-Verhältnis verringert das Lösungsvermögen des Pulvers, was zu größeren Partikeln, höheren Schubspannungen und stärkerer Scherverdünnung der Dispersion führt. Je nach Pulvertyp nahmen die Partikelgrößen und Schubspannungen wie folgt zu: SM < MPC50 < MC < MPC85. SM und MPC50 waren bereits nach 45 min bei 50 °C vollständig rehydriert. Im Gegensatz dazu benötigten Pulver mit hohem Proteingehalt wie MPC85 und MC einen HPH-Schritt bei 500 bar, um alle Pulveraggregate zu zerstören. Nach der vollständigen Rehydrierung der Pulver waren die Partikelgrößen und die Rheologie der rekonstituierten und frischen Konzentrate equivalent. Daraus schließen wir, dass die Partikelgröße der wichtigste Einflussfaktor für die rheologischen Eigenschaften ist. Unsere Ergebnisse zeigen, dass frische Konzentrate mit rekonstituierten Konzentraten hinsichtlich ihrer rheologischen Eigenschaften vollständig substituierbar sind, wenn die Rehydratationsbedingungen an den Pulvertyp angepasst werden.

Da Pulveraggregate die rheologischen Eigenschaften von rekonstituierten Konzentraten beeinflussen, wollten wir auch ihre Auswirkungen auf die Proteinfunktionalität bewerten. Zu diesem Zweck wurde das Gelierverhalten von MC88- und MPC85-angereicherter Magermilch untersucht. Wie bei der Käseherstellung üblich, wurde Magermilch für die Versuche entweder mit MC88 oder MPC85 auf eine Caseinkonzentration von 4,5 % (w/w) angereichert. Bewertungskriterien waren die Partikelgröße in Abhängigkeit von der Scherrate und die Labgeleigenschaften, d. h. Gelierzeit, Gelstärke, Strukturverlust bei Deformation und Serumverlust. Außerdem wurde die Casein-, Molkenprotein- und Caseinmakropeptid (CMP)-Rückgewinnung in der Süßmolke bestimmt, um die scher- und damit partikelgrößenabhängige Proteinverfügbarkeit zu bewerten. Da in der Molkereiindustrie zwei Hochschereinheiten weit verbreitet sind, können diese für die Redispergierung von Pulver verwendet werden: Scherpumpen wie in Pulvermischern und HPH. Daher führten wir zunächst Experimente im Labormaßstab durch, wobei wir eine Kolloidmühle als Rotor/Stator-System und einen HPH im Labormaßstab verwendeten. Für den anschließenden Scale-up wurden eine Scherpumpe und ein HPH im Pilotmaßstab verwendet.

Es stellte sich heraus, dass die Strömungsbedingungen in den Rotor/Stator-Systemen selbst bei Scherraten von bis zu $7{,}4 \times 10^4\ s^{-1}$ für eine vollständige Pulverlösung unzureichend waren; zusätzliche Kavitation, wie sie in HPH auftritt, war für eine vollständige Pulverauflösung erforderlich. Eine unzureichende Pulverrehydratation verlängert die Gelbildungszeit. Die Gelbildungszeit von MC88- und MPC85-angereicherter Magermilch war ähnlich; der Zusatz von MPC85 führte jedoch zu weicheren, schwächeren Gelen und folglich zu einer geringeren Synärese/ einem geringeren Serumverlust. Außerdem verringerten die verbleibenden Pulverpartikel die Molkenproteinkonzentration in der Süßmolke.

Bei der Käseherstellung fallen 70 % Süßmolke an, die in der Regel aufgereinigt wird, um die isolierten Molkenproteine zu gewinnen. In einigen Teilen der Welt, in denen es keine Infrastruktur für die Molkeverarbeitung gibt, muss die Molke jedoch verworfen werden, was eine potenzielle Umweltbelastung darstellt. Ein neuartiges Konzept zur Vermeidung des Molkeverlustes, das in dieser Arbeit untersucht wird, ist die Herstellung von Käse auf Pulverbasis mit hoher Ausbeute durch ein erhöhtes Casein/Molkenprotein-Verhältnis oder die Integration der Molkenproteine in die Käsematrix.

Untersucht wurde der Einfluss des Casein/Molkenprotein-Verhältnisses (86 : 14 und 98 : 2) und der Hitzebehandlung (80 °C/30 min) auf das Gelierverhalten von rekonstituierten Labgelen (d.h. den rekonstituierten, gelierten Konzentraten) und auf die Überlebensrate des integrierten *Lactobacillus paracasei* ssp. *paracasei* F19. Die Bewertungskriterien für die Labgeleigenschaften waren die Gelbildungsrate, die Gelfestigkeit und die Molkensynärese. Darüber hinaus wurden die Menge der integrierten Molkenproteinen und die resultierende Käseausbeute bewertet. Außerdem wurde die Lebendkeimzahl nach der Sprühtrocknung und während der 103-tägigen Lagerung des Pulvers bei 4, 10 und 20 °C bestimmt.

Die Gesamtproteinkonzentration wurde durch Ultrafiltration (UF) erhöht und das Casein/Molkenprotein-Verhältnis durch Mikrofiltration (MF) im Diafiltrationsmodus (DF) eingestellt. Konzentrierung wirkt sich jedoch zwangsläufig auf die Viskosität der Milch aus – insbesondere nach der Hitzebehandlung. Daher untersuchten wir auch die Mechanismen, die für die Veränderungen der Viskosität bei Erhöhung der Gesamtproteinkonzentration (3-18%), Variation des Casein/Molkenprotein-Verhältnisses (85 : 15, 92 : 8, 98 : 2) und Erhitzung (80 °C/30 min) verantwortlich sind. Unsere Ergebnisse zeigen, dass sowohl Erhitzung als auch Konzentration die Viskosität nicht zwangsläufig erhöhen. Ein erhitztes Konzentrat bleibt selbst bei hohen Gesamtpro-

teinkonzentrationen flüssig und leicht zu verarbeiten, wenn die Molkenproteine teilweise abgereichert werden (hier 37 %). Ist eine hohe Caseinkonzentration gewünscht, sollte die maximale Gesamtproteinkonzentration 9 % nicht überschreiten, um die Hydratation der Caseinmicellen und deren Abstoßungskräfte auf einem akzeptablen Niveau zu halten, bei dem die Viskosität noch niedrig und unbeeinflusst ist. Mit Hilfe eines statistischen Modells kann die scheinbare Viskosität von Milchproteinkonzentraten mit unterschiedlichen Gesamtproteinkonzentrationen und Casein/Molkenprotein-Verhältnissen berechnet und besser abgeschätzt werden, wie ausgeprägt die Unterschiede in der Viskosität nach dem Erhitzen sein werden. Dies sollte bei der Gestaltung des Instant-Käseprozesses helfen, bei dem die scheinbare Viskosität ein limitierender Faktor ist – insbesondere nach der Kaltverlabung.

Daher wurden die Gesamtproteinkonzentrationen bei der Herstellung der Instant-Käsepulver auf einem akzeptablen Niveau gehalten, um einen zu drastischen Viskositätsanstieg zu vermeiden. Hinsichtlich der Labgeleigenschaften zeigte sich, dass sich die Erhitzung positiv auf das Gelierverhalten des rekonstituierten UF-Pulvers auswirkte, da sowohl die Gelbildungsrate als auch die Gelfestigkeit bei gleicher Gesamttrockenmasse auf höhere Werte als bei den MF-Proben anstieg. Durch das höhere Serumbindevermögen des Bruchs ist dieses Pulver für Käsesorten mit höherem Feuchtigkeitsgehalt geeignet. Für Käsesorten mit geringerem Feuchtigkeitsgehalt sind die MF-Pulver aufgrund ihres engmaschigen Gelnetzwerks und der besseren Molkedrainage zu empfehlen. Da MF-Konzentrate ohnehin ein hohes Casein/Molkenprotein-Verhältnis aufweisen, kann auf eine Erhitzung im Hinblick auf die Labgeleigenschaften verzichtet werden. Allerdings wirkt sich die Erhitzung in beiden Proben unabhängig vom Casein/Molkenprotein-Verhältnis nach der Sprühtrocknung positiv auf die Lebendkeimzahl aus. Dies macht die Erhitzung zu einem geeigneten Werkzeug für die Funktionalisierung von Konzentraten und folglich von Pulvern, was darüber hinaus die Lebendkeimzahlen integrierter Bakterien erhöht. Wir konnten zeigen, dass die Käseausbeute steigt, wenn Pulver aus erhitzten UF-Konzentraten oder Pulver aus nicht erhitzten MF-Konzentraten verwendet werden.

Alle Aspekte zusammen betrachtet, wurden die Hypothesen dieser Arbeit bestätigt. Verbleibende Pulveraggregate erhöhen die Viskosität von rekonstituierten Magermilchkonzentraten im Vergleich zu frisch hergestellten Konzentraten gleicher Zusammensetzung. Darüber hinaus beeinträchtigen sie das Labgelierverhalten von proteinangereicherter Magermilch aufgrund der unzugänglichen Proteine in den Pulveraggregaten sowie die Geleigenschaften, da die Gele durch große Pulverpartikel gestört werden können.

Für die Viskositätsabschätzung von erhitzten, frisch hergestellten Milchproteinkonzentraten müssen die Wechselwirkungen zwischen Caseinmicellen und Molkenproteinen sowie die geometrische Anordnung der Proteine und Proteinkomplexe unterschiedlicher Größe berücksichtigt werden. Kaltverlabte UF- und MF-Pulver ergeben bei der Rehydratation bei Temperaturen unter 16 °C eine homogene Gelmatrix, sofern sie homogenisiert wurden. Durch die Konzentrierung können bis zu 87,2 % der denaturierten Molkenproteine integriert werden, ohne dass die Labgeleigenschaften beeinträchtigt werden. Grund hierfür sind die ausreichenden Mengen an kolloidalem Calcium und Calciumbrücken. Außerdem war die Überlebensrate in den erhitzten Konzentraten höher als in den unerhitzten, was auf die stärkere Denaturierung der Molkenproteine und die wahrscheinlich intensiveren hydrophoben Wechselwirkungen zwischen Bakterien und denaturierten Molkenproteinen zurückzuführen ist.

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Appendix

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Non-reviewed publications

Warncke, M. (2020): Oszillationsrheometrie zur Detektion der Labgelbildung. Milchwissenschaftliche Forschung Weihenstephan, Jahresbericht 2020, ISBN 978-3-947492-20-6

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