Heat Stability of Concentrated Milk Systems

Joseph Dumpler

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Kinetics of the Dissociation and Aggregation in High Heated Concentrated Milk Systems

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Dedicated to my students

Ever tried. Ever failed.
No matter.
Try again. Fail again.
Fail better.
(Samuel Beckett)

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'Acti iucundi labores' (Cicero), a statement which means 'Completed works are pleasant' when translated to English might be signed by every PhD candidate with regard to his or her finished PhD thesis.

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Contents

1 General introduction 1

1.1 Composition of milk and its implication on the heat stability 2

1.1.1 Caseins 3

1.1.2 Whey proteins 8

1.1.3 Lactose 10

1.1.4 Milk salts 13

1.2 Concentration of milk and high heat treatment 16

1.2.1 Principles of evaporation and reverse osmosis 17

1.2.2 Design of high heat treatments for dairy products 18

1.3 Methods for the determination of the heat stability 30

1.3.1 Subjective heat stability test methods 30

1.3.2 Objective determination of heat-induced coagulation 33

1.4 Factors that affect heat stability of milk and concentrated milk 35

1.4.1 Dependency of stability on pH and heating temperature 36

1.4.2 The effects of processing factors and additives 39

2 Motivation and objectives 45

3 Heat stability of concentrated skim milk on lab scale 47

Abstract 47

3.1 Introduction 48

3.2 Materials an methods 49

3.3 Results and discussion 52

3.4 Conclusion 59

Summary and contribution of the doctoral candidate 60

4 Heat stability of concentrated skim milk on pilot scale 63

Abstract 63

4.1 Introduction 64

4.2 Materials and methods ... 66
4.3 Results and discussion ... 70
4.4 Conclusion ... 79
Summary and contribution of the doctoral candidate ... 80
5 Dissociation and coagulation of caseins and whey proteins ... 83
Abstract ... 83
5.1 Introduction ... 84
5.2 Materials and methods ... 85
5.3 Results and discussion ... 89
5.4 Conclusions ... 100
Summary and contribution of the doctoral candidate ... 101
6 Preparation of simulated milk ultrafiltrate ... 103
Abstract ... 103
6.1 Introduction ... 104
6.2 Materials and methods ... 106
6.3 Results and discussion ... 112
6.4 Conclusions ... 120
Appendix ... 121
Summary and contribution of the doctoral candidate ... 121
7 Modelling of the heat stability of concentrated milk ... 123
Abstract ... 123
7.1 Introduction ... 124
7.2 Materials and methods ... 125
7.3 Results and discussion ... 129
7.4 Conclusions ... 138
Appendix ... 140
Summary and contribution of the doctoral candidate ... 141
8 The effect of calcium and pH on heat treated micellar casein ... 143
Abstract ... 143
Highlights ... 144
8.1 Introduction ... 144
8.2 Materials an methods ... 146
8.3 Results and discussion ... 151
8.4 Conclusions ... 162
Summary and contribution of the doctoral candidate ... 163
9 Overall discussion and outlook ... 165
9.1 Heat stability of concentrated milk ... 166

9.2 Mechanisms of the heat coagulation of casein micelles ... 169
9.3 Changes in heat stability induced by preheating, milk fat, and homogenisation ... 173
9.4 Conclusion and outlook ... 178
10 Supplementary material ... 181
10.1 Supplementary note I ... 181
10.2 Supplementary note II ... 182
10.3 Supplementary note III ... 183
References ... 185
Appendix ... 197
Peer reviewed publications ... 197
Non reviewed publications ... 198
Oral presentations ... 199
Poster presentations ... 200

List of figures

Fig. 1-1: Scanning electron micrographs and transmission electron micrographs of casein micelles in milk and concentrated milk. ... 5
Fig. 1-2: Schematic representations of the casein micelles in milk ... 7
Fig. 1-3: Ribbon type models showing the 3D-structure of α-la and of β-lg using the software package UCSF Chimera ... 8
Fig. 1-4: Schematic representation of the proposed pathways of the formation of heat-induced whey protein-κ-casein complexes in heated skim milk. ... 10
Fig. 1-5: Overview of the early Maillard reaction in milk leading to the Amadori product lactulosyllysine ... 12
Fig. 1-6: Schematic representation of selected degradation routes of the degradation of lactose in heated milk. ... 12
Fig. 1-7: The partition of calcium in skim milk ... 15
Fig. 1-8: Iso-effect lines for selected microbial inactivation levels, chemical, and physical changes ... 22
Fig. 1-9: Different types of continuous high heating systems using saturated steam or hot water as a heat transfer medium. ... 25
Fig. 1-10: Principle of operation of direct heating systems ... 26
Fig. 1-11: Patented steam injector having three annular steam inlets and an adjustable mandrel to control the velocity of the product flow ... 27
Fig. 1-12: Exemplary temperature-time profiles of direct (A) and indirect (B) UHT plants ... 29
Fig. 1-13: Copper plated thermostatic paraffin bath used for the subjective heat stability test of Davies and White (1966) ... 31
Fig. 1-14: Aggregated protein and non-protein nitrogen plus proteose peptone nitrogen produced in 'normal' separated bulk milk and milk from an individual cow after heating at 135 °C for various periods ... 34
Fig. 1-15: Heat coagulation time (HCT) vs. pH profile for type A milk, type B milk, concentrated milk at 20% non-fat total solids, and serum protein-free casein micelle dispersions heated at 140 °C ... 37
Fig. 1-16: The relation between the logarithm of coagulation time and temperature of four different types of milk ... 37

Fig. 1-17: Processing alternatives for the manufacture of evaporated milk or concentrated sterile milk in general using the UHT process for sterilisation 39
Fig. 1-18: HCT vs. pH profile for concentrated milk at 20% non-fat total solids prepared from either preheated or non-preheated milk heated at 120 °C 40
Fig. 3-1: Assembly used for determination of heat stability of unconcentrated and concentrated milk of different total solids content 51
Fig. 3-2: Heating curves of individual milk samples subjected to heat stability test 52
Fig. 3-3: Heat coagulation time as a function of the pH at 20 °C 53
Fig. 3-4: Heat coagulation temperature as a function of the pH at 20 °C 54
Fig. 3-5: Heating curves of samples at various oil bath temperatures 55
Fig. 3-6: Correlation between milk sample temperature and time of coagulation of concentrated skim milk of different total solids content 56
Fig. 3-7: Heat coagulation time correlated with sample temperature of individual skim milk samples over a 15 months period 58
Fig. 4-1 Simplified P&I diagram of the pilot plant unit for direct steam injection heat treatment of concentrated skim milk 66
Fig. 4-2: Temperature-time profiles of the direct steam injection UHT pilot plant 68
Fig. 4-3: Scanning electron micrographs of protein particles formed during direct steam injection heat treatment of concentrated skim milk at 145 °C 71
Fig. 4-4: Scanning electron micrographs of supernatants of concentrated skim milk 72
Fig. 4-5: Logarithmic density distribution measured by laser light diffraction of concentrated skim milk heat treated by direct steam injection at different temperatures 73
Fig. 4-6: Logarithmic density distribution of the supernatant from preheated concentrated skim milk heat treated by direct steam injection at different temperatures 73
Fig. 4-7: Sedimentable aggregated protein of concentrated skim milk 76
Fig. 4-8: Iso-effect lines of the onset of coagulation of concentrated skim milk heated by direct steam injection plotted on a semi-logarithmic scale 78
Fig. 5-1: Sample preparation procedure for separation of the different particle fractions and RP-HPLC analysis 86
Fig. 5-2: Logarithmic density distributions of CSM heat treated at different temperatures 90
Fig. 5-3: Average volume-based particle diameter $d_{50,3}$ as a function of temperature, total solids, and holding time of DSI heat treatments 92
Fig. 5-4: RP-HPLC chromatograms of supernatants (4,000xg/10 min) of caseins and whey proteins in guanidine buffer 93

Fig. 5-5: Relative amount of non-sedimentable caseins in CSM at 27% total solids centrifuged at 4,000*x*g for 10 min heated at different temperatures 94
Fig. 5-6: RP-HPLC chromatograms of ultracentrifugal supernatants of caseins and whey proteins in guanidine buffer 95
Fig. 5-7: Relative amount of non-sedimentable caseins in CSM at different total solids after centrifugation and ultracentrifugation 96
Fig. 5-8: Relative amount of non-sedimentable whey proteins in CSM at different total solids after centrifugation and ultracentrifugation 98
Fig. 5-9: Relative amount of soluble β–casein and soluble κ-casein in the ultracentrifugal supernatant after heat treatment of concentrated skim milk of 27% total solids at different temperatures 99
Fig. 5-10: Schematic representation of the proposed model of heat-induced dissociation and coagulation of caseins and whey proteins in CSM 100
Fig. 6-1: High performance ion exchange chromatograms of ultrafiltration permeate obtained at 50 °C and simulated milk ultrafiltrate analysed for cations and anions 108
Fig. 6-2: Ca^{2+}-activity over pH for SMUF solutions in comparison to UF permeate 119
Fig. 7-1: Relative amount of sedimentable protein over the logarithmic heating time depending on heating temperature and total solids fitted by the Weibullian model 130
Fig. 7-2: Variation of the parameter *a* of the Weibullian model depending on total solids 131
Fig. 7-3: Parameter β_w of the Weibullian model obtained by non-linear regression of sedimentable protein depending on heating temperature and total solids content 132
Fig. 7-4: Arrhenius-plot of the parameter α_w of the Weibullian model obtained by non-linear regression of sedimentable protein depending on heating temperature of CSM 133
Fig. 7-5: Simulation of the relative amount of non-sedimentable protein depending on CSM total solids at 116 °C and 27% CSM total solids depending on heating temperature over time on a logarithmic scale 135
Fig. 7-6: Iso-effect lines for CSM for a critical level of 2% of sedimentable protein of total protein depending on heating temperature, heating time, and total solids content of bulk CSM 136
Fig. 7-7: Relative amount of soluble κ-casein and α_{S1}-casein depending on heating temperature at 27% CSM total solids over time 138
Fig. 7-8: Regular residuals of the Weibullian model fitted to the data of relative amounts of sedimentable protein of total protein and the normal probability plots of the residuals. 140
Fig. 8-1: Amount of whey proteins in the MF retentate of during concentration and DF of milk with simulated milk ultrafiltrate 147

Fig. 8-2: Decrease in pH during heat treatment of MCC at different temperatures and the corresponding pH after dilution of MCC with SMUF buffer 152
Fig. 8-3: Relative amount of dissociated κ-casein, α_{S2}-casein, α_{S1}-casein, and β-casein analysed in the supernatant of MCC ultracentrifuged at 48,800*x*g/26 min after heating at different temperatures 153
Fig. 8-4: Relative amount of dissociated κ-casein and α_{S1}-casein in MCC analysed in the supernatant of MCC ultracentrifuged at 48,800*x*g/26 min after heating at a temperature of 116 °C at different pH 155
Fig. 8-5: Heat coagulation temperature and heat coagulation time of MCC depending on pH and addition of soluble calcium 156
Fig. 8-6: Calcium activity in MCC depending on pH and addition of calcium as calcium chloride 157
Fig. 8-7: Relative amount of dissociated κ-casein and α_{S1}-casein in MCC analysed in the ultracentrifugal supernatant of MCC after heating at a temperature of 116 °C and addition of soluble calcium 157
Fig. 8-8: Volume based average diameter $d_{50,3}$ of casein micelles in heat treated MCC at different temperatures, different pH, diffent calcium levels and ionic strength 159
Fig. 8-9: The maximum $d_{50,3}$ of heat treated MCC depending on pH and soluble calcium level 160
Fig. 8-10: Relative amount of dissociated κ-casein and α_{S1}-casein in MCC analysed in the ultracentrifugal supernatant and centrifugal supernatant after heating at temperature of 116 °C, pH adjustment to 6.35, and addition of 3 mM soluble calcium 161
Fig. 9-1: Correlation between milk sample temperature and time of coagulation of concentrated skim milk of different total solids content 167
Fig. 9-2: Iso-effect lines for CSM for a critical level of 2% of sedimentable protein of total protein depending on heating temperature, heating time, and total solids content of bulk CSM 168
Fig. 9-3: Visually determined heat coagulation temperature and heat coagulation time of concentrated skim milk on lab scale depending on the total solids content 175
Fig. 9-4: Visually determined heat coagulation temperature and heat coagulation time of concentrated milk containing various proportions of milk fat depending on the total solids content 176
Fig. 9-5: Visually determined heat coagulation temperature and heat coagulation time of concentrated milk containing various proportions of milk fat depending on volume corrected non-fat total solids 177
Fig. 10-1: Calculation of the expected relative amount of sedimentable protein based on the kinetic data for direct steam injection heat treatments 181

Fig. 10-2: Iso-effect lines for CSM for a critical level of 5% and 10% of sedimentable protein of total protein depending on heating temperature, heating time, and total solids content of bulk CSM 183
Fig. 10-3: Calculated data for the factor f_T depending on temperature and f_s depending on total solids content of CSM 184

List of tables

Tab. 1–1: Composition of one kilogram of bovine milk 3
Tab. 4–1: The pH values of skim milk and concentrated skim milk (CSM) before heat treatment 67
Tab. 4–2: Parameters of the Gompertz equation fitted to the data of sedimentable protein after DSI heat treatment of CSM of various total solids content 76
Tab. 4–3: Parameters of the Gompertz equation of different holding times at 27, 23% total solids, and at 27% totals solids of UHT preheated milk 77
Tab. 6–1: Quantities of individual salts needed for the preparation of a SMUF simulating ultrafiltration permeate at 10 °C and 50 °C 109
Tab. 6–2: Ionic composition of ultrafiltration permeate obtained at different filtration temperatures 114
Tab. 6–3: Physical properties, dry matter and lactose content of ultrafiltration permeate obtained at different filtration temperatures 115
Tab. 6–4: Total ionic composition of different milk samples determined by precipitation of proteins and solubilisation of colloidal salts 115
Tab. 6–5 Calculated amounts of individual ionic species of different SMUF solutions; comparison with the SMUF solutions of Jenness and Koops (1962) 116
Tab. 6–6 Ionic composition of different SMUF solutions as determined by ion chromatography 117
Tab. 6–7: Physical properties of different SMUF solutions with and without addition of lactose 118
Tab. 7–1: Parameters and calculated parameters derived from linear regression of the Arrhenius-plot of the parameter α_w in CSM at 27% total solids 134
Tab. 7–2: Parameters and calculated parameters derived from linear regression of the plot of the parameter α_w depending on total solids content of CSM at a heating temperature of 116 °C 134
Tab. 8–1: Composition of the MCC powder 148
Tab. 8–2: Physical characteristics and protein composition of the reconstituted MCC 150
Tab. 10–1: Comparison of the observed and calculated temperatures of the onset of coagulation 182

Abbreviations

Latin symbols

A	Membrane constant	m^2 s kg^{-1}
A	Constant	0.51
AMP	Advanced Maillard products	
BLE	Federal Office for Agriculture and Food (Germany)	
BMEL	Federal Ministry of Food and Agriculture (Germany)	
BSA	Bovine serum albumine	
c	Concentration	mol L^{-1}, M
C5, C6	Molecule with five and six carbon atoms in the backbone, respectively	
C_0	Initial concentration (of protein)	g L^{-1}, %
C_∞	Concentration of protein after infinite heating time	%
CCP	Colloidal calcium phosphate	
CD	Circular dichroism	
CSM	Concentrated skim milk	
C_t	Concentration at time t	g L^{-1}
$C_{T,t}$	Concentration of protein at a discrete temperature and holding time	%
DD	Degree of denaturation	
DSI	Direct steam injection	
D_ϑ	Decimal reduction time at temperature ϑ	s
E_A	Activation energy	kJ mol^{-1}
EDTA	Ethylenediaminetetraacetic acid	
ESL	Extended shelf life	
FAO	Federal Agriculture Organisation	
FMP	Final Maillard product	
f_s	Scaling factor for α_W depending on CSM total solids	-
f_T	Scaling factor for α_W depending on temperature	-
FTIR	Fourier transform infrared spectroscopy	

gal	Galactose	
HCl	Hydrochloric acid	
HCT	Heat coagulation time	s, min
HPLC	High performance liquid chromatography	
I	Ionic strength	mol L^{-1}
J	Flux	m^3 m^2 s
k	Rate constant	s^{-1}
k	Gompertz parameter	$°C^{-1}$
k_0	Apparent rate constant at $\lim_{\frac{1}{T}\to 0} k_T$	s^{-1}
k_{app}	Apparent rate constant	s^{-1}
k_B	Boltzmann constant	$1.3806 \cdot 10^{-23}$ J K^{-1}
KCl	Potassium chloride	
KOH	Potassium hydroxide	
k_{ref}	Rate constant at reference state	s^{-1}
k_T	Rate constant at temperature T	s^{-1}
LOD	Limit of detection	
LOQ	Limit of quantification	
MCC	Micellar casein concentrate	
MPC	Milk protein concentrate	
n	Reaction order	-
N_0	Initial number of microorganisms	-
N_A	Avogadro constant	$6.0221 \cdot 10^{-23}$ mol^{-1}
NaOH	Sodium hydroxide	
NPN	Non-protein nitrogen	
N_{res}	Number of resistant microorganisms	-
N_t	Number of microorganisms at time t	-
P&I	Pipe and instrumentation	
PCS	Photon correlation spectroscopy	
PDB	Protein data base	
PEEK	Polyether ether ketone	
P_i	Inorganic phosphate	
PI_u	Upper limit of the (95%) probability interval	
PPN	Proteose peptone nitrogen	
PS-DVB	Polystyrene divinylbenzene	
Q_{10}	Q_{10}-value	K; °C
R	Universal gas constant	J mol^{-1} K^{-1}
R_1, R_2	Organic residues	
R^2	Coefficient of determination	-
RO	Reverse osmosis	
RP-HPLC	Revesed-phase high performance liquid chromatography	
s	Total solids content of concentrated skim milk	%
S(t)	Survival ratio at time t	-

SMUF	Simulated milk ultrafiltrate	
SMUF-JK	Simulated milk ultrafiltrate according to Jenness and Koops (1962)	
S_P	Sedimentable protein	%
$S_{P,max}$	Maximal amount of sedimentable protein	%
s_{ref}	Reference total solids content of CSM	%
T	Absolute temperature	K
T	Temperature of the Gompertz model	°C
T^*	Reference temperature plus 10 K	K
T_c	Temperature of the inflection point (Gompertz model)	°C
TFA	Trifluoroacetic acid	
thermoph.	thermophilic	
t_R	Reliable lifetime	s
T_{ref}	Temperature at reference state	K
t_{ref}	Reference time	s
TS_0	Initial total solids content	%
$TS_{non\text{-}fat}$	Non-fat total solids content	%
$TS_{non\text{-}fat,\ vc}$	Volume corrected non-fat total solids content	%
TS_T	Total solids content at temperature T	%
UF	Ultrafiltration	
UHT	Ultra-high temperature	
v	Reaction velocity	$s^{-1}\ M^n$
vc	Volume corrected	
VRF	Volume reduction factor	
w/v	Weight per volume	
z	*z*-value	K; °C
z_i	Valency of an ion	-

Greek symbols

$\mu_{\alpha_{w,T}}$	Slope of the regression line of the Arrhenius plot for the characteristic time α_W at temperature T	K
α	activity	$mol\ L^{-1}$
α-la	α-Lactalbumin	
α_W	Weibull parameter	s^{-1}
β-lg	β-Lactoglobulin	
β_W	Weibull parameter	-
γ_i	activity coefficient	-
Δp	Pressure difference	Pa
Δp_{DL}	Presure difference of a deposit layer	Pa

$\Delta\pi$	Osmotic pressure difference	Pa
ϑ	temperature	°C
ϱ_{CSM}	Density of concentrated skim milk	kg m^{-3}
ϱ_{fat}	Density of milk fat globules	kg m^{-3}
Φ_{fat}	Volume fraction of milk fat globules	-

Summary

The necessity to determine the heat stability of concentrated milk originates from the manufacture of evaporated milk and dates back to the late 19th century. It became an issue due to observed particle formation, gelation, and sediment formation in evaporated milk during sterilisation and subsequent storage. Certain batches of evaporated milk, sterilised in cans or glass, and later in HD-PE bottles, showed these instabilities during heat treatment and therefore became defective. Hence, there was an increasing economic interest in the basic understanding and control of the heat stability of the concentrated milk. Since then, dairy science deals with the topic of the heat stability of milk and especially concentrated milk concerning factors affecting the heat stability, methods to predict, and measures to improve it. However, the mechanism of coagulation, factors that cause coagulation and modelling approaches to predict the heat stability of concentrated milk for process optimisation were still lacking.

Individual milieu conditions that affect the heat stability of milk or milk concentrated by evaporation or reverse osmosis have been intensively investigated in the past. These factors comprise the initial pH before heating, the amount of soluble divalent cations, especially soluble calcium, the amount of reactive whey proteins, and the ionic strength in the milk serum. The heat stability of unconcentrated bulk milk at its natural pH is maximal in most cases when dilution is not considered. Attempts to compensate for changes that occur during concentration of milk by removal of water, i.e. changes in pH, ionic strength, soluble calcium, and protein content could not restore the heat stability of unconcentrated milk. Attempts made comprise the addition of bases, citrates, phosphates, and many other permitted and illegal additives for practical applications and research, respectively. Other membrane techniques using porous membranes such as nano-, ultra-, and microfiltration also lead to a reduction in the heat stability of the final concentrate as compared to the unconcentrated milk. However, the overall heat stability of the concentrate increases with increasing compositional similarity of the concentrate to unconcentrated milk. This observation seems plausible. Nevertheless, a prediction of the heat stability of these concentrates in terms of maximum temperature-time combinations for these concentrates under continuous heating conditions without coagulation was lacking.

Technological treatments such as treatments of milk before concentration and heat treatment can affect the heat stability of the final concentrate positively or negatively. Therefore, the combination of processing steps and the choice of processing parame-

ters is decisive to maintain or improve the heat stability of the concentrate. Homogenisation of milk or concentrated milk before heat treatment of the concentrate negatively affects the heat stability. Appropriate preheating of the milk before concentration increases the heat stability of the concentrate. A quantitative estimation of the heat stability expressed as a shift in critical temperature-time combinations had not been performed.

Therefore, the aim of this study was to establish a laboratory test method and a model able to describe the heat stability of concentrated skim milk of various total solids under continuous heating conditions. The limited heat stability of concentrated skim milk of various total solids content should be described quantitatively on lab- and pilot scale. The intention was to be able to design heating processes that maximise microbial inactivation in the concentrates by, at the same time, avoiding heat-induced coagulation of the concentrates. In addition, a description of selected heat-induced changes on casein micelles was also intended. Explanations for the decrease in heat stability by concentration of milk due to changes in milieu conditions should be found and attributed to single components. It was assumed that certain milieu conditions increase the rate of heat-induced changes on casein micelles reducing their colloidal stability and thereby induce coagulation.

The investigation of the heat stability on lab scale was performed using small amounts of concentrated milk filled into small glass tubes tightly screwed and immersed into an oil bath for heating until visual coagulation was observed. Heat coagulation time and temperature were recorded as many samples readily coagulated during heating-up times. The aim of the investigations was to determine the effect of pH, total solids content, preheat treatments, and milk fat on critical temperature-time combinations of the onset of coagulation on lab scale. These critical temperature-time combinations for different total solids content were established by varying the oil bath temperature, i.e. the heating intensity of the samples. The investigation showed that the heat stability of the concentrates progressively decreases with increasing total solids content. A correlation of the heat stability of bulk milk and concentrates produced thereof was possible. The influence of the pH on heat stability decreased with increasing total solids content of the concentrates. The heat stability maximum was at pH 6.7 in all cases. A linear relationship between the logarithm of the coagulation time and the heat coagulation temperature could be established for discrete total solids contents of CSM. This indicated that heat-induced coagulation of concentrated milk follows the principles of formal reaction kinetics. However, an immediate transfer of coagulation temperatures and times to pilot and industrial scale was not possible as the heating profiles of the lab scale system and continuous heating were different.

Direct steam injection was used to perform heat stability testing on pilot scale as it enables isothermal heat treatment of concentrated milk (15-31.5% total solids) due to negligible come-up and cooling times. It was possible to determine the onset of coagulation and the course of coagulation over temperature (117-153 °C) of concentrated skim milk of different total solids content at short holding times (< 13 s). A rapid

increase in the amount of sedimentable heat-induced protein aggregates was observed when certain critical temperature-time combinations were exceeded. These aggregates formed from casein micelles had a size of 3-20 µm, although secondary aggregation to particles of 10-100 µm was pronounced. The size of the aggregates enabled their quantitative separation at 4,000*x*g/10 min in concentrated skim milk. These trials showed a clear correlation between the maximum heating intensity in terms of heating temperature and holding time and the dry matter of the concentrates. A UHT preheat treatment of the milk before concentration resulted in an increase in the heat stability of the concentrate. Higher temperatures or longer holding times became possible without formation of casein micelle aggregates. Heating intensities without coagulation resulted in a remarkable increase in casein micelle size in heated concentrated skim milk. In conjunction with the formation of dissociated casein particles in the range of 20-100 nm, this indicates a marked structural disintegration of casein micelles as a consequence of the heat treatment. This loss of structural integrity of casein micelles could be a preceding reaction to heat-induced coagulation of casein micelles.

Therefore, structural changes of casein micelles and the kinetics of heat-induced aggregation of casein micelles needed to be further addressed. Quantitative ultracentrifugal separation of the different size fractions in direct steam injection heat treated concentrated skim milk was applied. The quantitative separation of the different size fractions was monitored by particle sizing techniques. At the same time, changes in particle size of casein micelles in heated concentrated skim milk of different total solids content were also investigated. The obtained size fractions were analysed for their relative amounts of individual caseins and whey proteins by a refined RP-HPLC method from literature to obtain mechanistic insights into the course and mechanism of heat-induced coagulation of casein micelles. The results showed an accelerated increase of casein micelles with increasing total solids content of the concentrates and increased heating temperature as it was observed for heat-induced coagulation. Coagulated casein was formed by calcium sensitive α_S- and β-caseins. Increasing the heating intensity increased the amount of dissociated casein, mainly κ-casein. Coagulation of casein micelles was observed when 30-35% of κ-casein had dissociated. In addition, the size of casein micelles had doubled at that point. It is assumed that more than one reaction contributes to the destabilisation of casein micelles leading to coagulation.

Furthermore, an extension of the heat treatment trials of concentrated milk and the knowledge obtained concerning the relationship of heating temperature, holding time, and total solids content of the concentrate was intended to derive kinetic parameters (rate constants, activation energies) of heat-induced aggregation of casein micelles. For this purpose, stainless steel tubes were filled with concentrated skim milk (12-33% total solids) and indirectly heated with saturated steam at temperatures from 103 to 131 °C for 0 to 5000 s. After heat treatment, coagulated casein particles were removed by centrifugation. The course of the coagulation process at 27% total solids heated at different temperatures as well as the concentrates of different total

solids content heated at 116 °C could be describe by a Weibullian model. Using this model, critical limits for the heat treatment of concentrated skim milk of different total solids content could be defined. The temperature dependency of the reaction rate could be derived. A basis for the design of non-isothermal heat treatment of concentrates by continuous integration of temperature-time effects was established. The validation of the model was performed by the data obtained from direct steam injection heat treatments at higher temperatures and shorter holding times.

A comparison of the heat stability test results on lab scale with critical temperature-time combinations using direct steam injection heating on pilot scale as well as data obtained by reaction kinetic calculations was performed. This comparison enables to determine the heat stability of different milk concentrates on lab scale and to predict the heat stability of the concentrates under continuous heat treatment.

Further insights into the mechanism of heat-induced coagulation and the relation of the heat-induced dissociation of κ-casein, the increase in casein micelle size, and the heat-induced aggregation of casein micelles in the complex system of concentrated milk appeared to be difficult. Hence, these mechanistic aspects were assessed by using a model system of micellar casein as obtained by diafiltration. This whey protein- and lactose-free casein micelle suspension was manufactured by multiple diafiltration using a simulated milk ultrafiltrate (SMUF). SMUF is a synthetic salt solution that closely resembles the natural milk serum composition. This SMUF solution was developed based on the results of the analysis of ultrafiltration permeate by analytical high-performance ion chromatography which was established for this purpose. In addition, the salt composition and the physical-chemical properties of the SMUF solution depending on temperature and pH were thoroughly characterized. An identical composition and the similarity of the main physico-chemical properties, especially at different working temperatures without crystallisation of calcium phosphate at different working temperatures could be achieved. The dependency of the calcium activity on SMUF composition and pH could be determined by a calcium selective electrode. The region of supersaturation of the SMUF could be predicted by the determination of the pH-dependency of the calcium activity in skim milk.

This diafiltered casein micelle model system using SMUF as a diafiltration medium facilitated a targeted modification of milieu conditions. The interference of the Maillard reaction with analytical determination of caseins by RP-HPLC and the mechanism of heat-induced coagulation was minimised. An increased rate of κ-casein dissociation due to thiol-disulphide exchange reactions with β-lactoglobulin was prevented. The investigations into the effect of pH on the heat-induced coagulation of micellar casein showed that at pH > 6.7 coagulation of casein was very limited, whereas the dissociation of casein, especially κ-casein, from the micelles was very pronounced. With increasing temperature, the amount of dissociated casein increased at the same heating time. Gradual decrease in pH decreased the amount of dissociated casein over holding time at 116 °C. However, there was a significant increase in casein micelle size that could also be observed when soluble calcium was added. The additional increase in ionic strength after calcium addition and a reduc-

tion in pH induced the coagulation of casein micelles to distinct particles. This indicates that in the pH-range of 6.2-7.2, several factors destabilising casein micelles must be present to reduce the colloidal stability of casein micelles and to induce heat-induced coagulation of casein micelles to particles. These factors comprise an increased amount of soluble calcium, increased ionic strength, and a particular heating intensity which are all present in heated concentrated skim milk. Low pH and high soluble calcium content result in the loosening of the internal structure of the casein micelles. The newly-created calcium-sensitive surface of the casein micelles facilitates the aggregation of the casein micelles by exposure of calcium sensitive caseins on the surface of the micelles. The dissociation of κ-casein does not appear to be directly related to heat-induced aggregation, especially at pH < 6.7. However, it should be regarded as a deviation from the original micellar structure of the casein micelles in unheated milk at its natural pH that affects the physical properties of the casein micelles and is likely to foster heat-induced aggregation of casein micelles.

An extension of the term 'heat stability' of the casein micelles is likely to be necessary. A consideration of the dissociation of caseins as well as the loosening of the internal structure of the casein micelles, both related to a loss in native structure is necessary.

The key outcomes of this work can be summarized as follows.

- A kinetic description of heat-induced coagulation of concentrated skim milk on lab- and pilot scale for the determination and calculation of critical temperature-time combinations without coagulation of the concentrates.
- Studies on the dissociation and coagulation of individual caseins in concentrated skim milk heated by direct steam injection.
- The development of a simulated milk ultrafiltrate, e.g. for the purification of casein micelles, to investigate targeted changes in serum composition on heat-induced changes of casein micelles.
- Investigations on heat-induced changes in casein micelle structure and dissociation of caseins in heat treated micellar casein depending on ionic strength, pH and soluble calcium.

Zusammenfassung

Die Notwendigkeit der Bestimmung der Hitzestabilität konzentrierter Milch nahm ihren Anfang mit der industriellen Herstellung von Kondensmilch im späten 19. Jahrhundert. Die Thematik war aufgrund beobachteter Probleme mit der Gerinnung, Sedimentbildung und damit Unbrauchbarkeit einzelner Chargen konzentrierter Milch bei der Sterilisation in Dosen, später in Glas- und HD-PE-Flaschen, von großem wirtschaftlichem Interesse. Seither beschäftigte sich die Milchwissenschaft mit der Thematik der Hitzekoagulation von Milch, insbesondere konzentrierter Milch, deren Einflussfaktoren, Methoden zur Vorhersage und der Verbesserung der Hitzestabilität. Dabei sind trotz intensivster Forschungsarbeit bis heute noch viele Fragen zum Mechanismus, den auslösenden Faktoren der hitzeinduzierten Aggregation der Caseinmicellen und der Modellierung der Reaktion im Sinne der Prozessoptimierung offen.

Die einzelnen Millieufaktoren, die die Hitzestabilität von Milch bzw. mittels Eindampfung oder Umkehrosmose konzentrierter Milch bestimmen, sind sehr intensiv untersucht worden. Zu diesen Faktoren gehören insbesondere der pH-Wert vor der Erhitzung, der Gehalt an löslichen divalenten Kationen im Milchserum, vorwiegend lösliches Calcium, der Gehalt an reaktiven Molkenproteinen und die Ionenstärke im Milchserum. Dabei ist unkonzentrierte Milch mit natürlichen pH-Wert meist am hitzestabilsten, wenn eine Verdünnung nicht in Betracht gezogen wird. Trotz des Versuches, die sich während der Konzentrierung von Milch ändernden Millieufaktoren pH-Wert, Ionenstärke, löslichen Calcium, Proteingehalt teilweise durch Zugabe von Basen, Citrat, Phosphat und vielen weiteren Zusatzstoffen auszugleichen, bleibt die Hitzestabilität konzentrierter Milch im Vergleich zum unkonzentrierten Zustand geringer. Auch andere Membranverfahren der Konzentrierung wie Nano-, Ultra- und Mikrofiltration führen zu einer Verringerung der Hitzestabilität. Dabei wird die Hitzestabilität des Konzentrates umso höher, je weniger dessen Zusammensetzung von der unkonzentrierten Milch abweicht. Dies erscheint plausibel, war jedoch bisher nicht quantitativ in Bezug auf die mögliche Erhitzungsintensität als Funktion der maximalen Temperatur-Zeit-Bedingungen für Konzentrate unter Bedingungen in Durchlauferhitzern ohne Koagulation beschreibbar.

Technologische Einflussfaktoren, d.h. vor allem die Verfahrensschritte vor der Konzentrierung der Milch und Erhitzung des Konzentrates, können sich sowohl negativ als auch positiv auf die Hitzestabilität des Konzentrates auswirken. Die Kom-

bination der einzelnen Verfahrensschritte und die Wahl der Prozessparameter sind daher sehr entscheidend bei der Herstellung haltbarer flüssiger Milchkonzentrate. Die Homogenisierung der Milch oder des Konzentrates vor der Erhitzung des Konzentrates wirkt sich negativ auf die Hitzestabilität aus. Im Falle einer geeigneten Vorerhitzung der Milch vor der Konzentrierung kann die Hitzestabilität gesteigert werden. Eine quantitative Aussage in Form der Verschiebung kritischer Temperatur-Zeit-Bedingungen war bisher nicht möglich. Zudem sind die Prozessparameter zur kontinuierlichen Erhitzung von Milchkonzentraten in Platten- oder Röhrenwärmeübertragern oder mittels direkter Erhitzungsverfahren bisher meist nur empirisch ermittelt und konnten nicht aus der im Labormaßstab ermittelten Hitzekoagulationszeit des Konzentrates ermittelt werden. Eine Berechnung möglicher Temperatur-Zeit-Bedingungen basierend auf reaktionskinetischen Parametern, wie dies für mikrobiologische Inaktivierungs- sowie chemische Effekte möglich ist, war bisher nicht möglich.

Daher war es das Ziel der hier vorliegenden Arbeit, einen Hitzestabilitätstest und eine Berechnungsgrundlage zu schaffen, durch die sich die Hitzestabilität von Milchkonzentraten unter kontinuierlichen Erhitzungsbedingungen, insbesondere von Magermilchkonzentraten, die mittels Umkehrosmose hergestellt wurden, beschreiben lässt. Die begrenzte Hitzestabilität von Magermilchkonzentraten unterschiedlicher Trockenmasse in Bezug auf Erhitzungstemperatur und -zeit sollte quantitativ im Labor- als auch im Technikumsmaßstab beschrieben werden. Dadurch sollte es möglich sein, Erhitzungsprozesse in Bezug auf erwünschte mikrobiologische Inaktivierungseffekte zu optimieren und die Koagulation der Konzentrate während der Erhitzung zu verhindern. Die Beschreibung ausgewählter hitzeinduzierter Veränderungen an den Caseinmicellen war ebenfalls ein Ziel dieser Arbeit. Daraus sollten Erklärungsansätze für die Verringerung der Hitzestabilität von Milch durch die Veränderung verschiedener Millieufaktoren während der Konzentrierung gefunden werden. Es wurde vermutet, dass bestimmte Millieufaktoren zu einer Beschleunigung der hitze-induzierten Verringerung der kolloidalen Stabilität der Caseinmicellen führen.

Die Untersuchungen zur Hitzestabilität im Labormaßstab erfolgte durch die Erhitzung von mit Magermilchkonzentrat befüllten Probengefäßen im Ölbad und der visuellen Bestimmung des Beginns der Koagulation der Konzentrate. Dabei wurde jeweils die Zeit bis zur einsetzenden Koagulation als auch die momentane Temperatur der Konzentrate registriert, da viele Konzentrate bereits während der Aufheizphase koagulierten. Das Ziel der Untersuchung der Hitzestabilität im Labormaßstab war es, den Einfluss von pH-Wert, fettfreier Trockenmasse, Vorerhitzung und Fettgehalt auf die sichtbare Koagulation im Labor-Erhitzungssystem zu bestimmen. Durch die Variation der Ölbadtemperatur sollte der Zusammenhang zwischen der Hitzekoagulationstemperatur und –zeit und diskreter Trockenmassen gefunden werden.

Die Analyse der Hitzestabilität im Labormaßstab ergab zunächst, dass die Hitzestabilität mit ansteigender Trockenmasse der Magermilchkonzentrate kontinuierlich

abnimmt. Eine Korrelation der Hitzestabilität von Sammelmilch und daraus hergestellter Konzentrate war möglich. Der pH-Wert der Konzentrate hatte mit steigender Trockenmasse einen geringer werdenden Einfluss auf die Hitzestabilität, wobei das Stabilitätsmaximum aller Trockenmassen bei einem pH-Wert von 6,7 lag. Zwischen der Koagulationstemperatur und dem Logarithmus der Hitzekoagulationszeit ließ sich für diskrete Trockenmassen ein linearer Zusammenhang darstellen. Dies deutete darauf hin, dass die hitzeinduzierte Koagulation von konzentrierter Milch reaktionskinetisch beschreiben lässt. Eine direkte Übertragbarkeit der kritischen Temperatur-Zeit-Bedingungen vom Labor- in den Pilot- und Industriemaßstab war aufgrund der unterschiedlichen Aufheizprofile des Labor-Erhitzungssystems und einer kontinuierlichen Anlage nicht möglich.

Zur Untersuchung der Hitzestabilität im Pilotmaßstab wurde die Direkterhitzung mittels Dampfinjektion verwendet, die es ermöglicht, bei vernachlässigbaren Aufheiz- und Abkühlzeiten die Konzentrate isotherm zu erhitzen. Damit konnten die Koagulationspunkte sowie der Koagulationsverlauf für unterschiedliche Trockenmassen und kurze Heißhaltezeiten (< 13 s) in Abhängigkeit der Erhitzungstemperatur (117-153 °C) bestimmt werden. Das Überschreiten bestimmter kritischer Temperatur-Zeit-Bedingungen führte zu einem starken Anstieg sedimentierbarer hitzeinduzierter Proteinaggregate. Diese Aggregate aus noch deutlich erkennbaren Caseinmicellen hatten etwa eine Größe von 3-20 µm, wobei die Sekundäraggregation zu Partikeln von 10-100 µm ausgeprägt war. Dadurch waren die hitzeinduzierten Proteinaggregate bereits bei 4000*x*g/10 min vollständig in den erhitzten Konzentraten sedimentierbar. Eine sehr deutliche Korrelation zwischen der maximalen Erhitzungsintensität als Funktion der Temperatur und Zeit und der Trockenmasse der Konzentrate konnte auch im Pilotmaßstab gezeigt werden. Durch eine UHT-Vorerhitzung der Magermilch vor der Konzentrierung konnte die Hitzestabilität der Konzentrate deutlich gesteigert werden, sodass höhere Temperatur-Zeit-Bedingungen zur Erhitzung ohne die Bildung von Proteinaggregaten möglich waren. Erhitzungsintensitäten ohne Koagulation führten zu keiner Sedimentbildung, jedoch bereits zu einer deutlichen Vergrößerung der Caseinmicellen im erhitzten Magermilchkonzentrat. Zusammen mit der Bildung von dissoziierten Caseinpartikeln im Bereich von 20-100 nm deutet dieses auf einen deutlichen Strukturverlust der Caseinmicellen infolge der Erhitzung hin, der die Vorreaktion zur Koagulation der Caseinmicellen darstellen könnte.

Die strukturellen Veränderungen an den Caseinmicellen sowie die Reaktionskinetik der Caseinaggregation sollten daher noch näher untersucht werden. Dazu wurde differentielle Zentrifugation zur quantitativen Trennung der einzelnen Größenfraktionen nach der Erhitzung der Magermilchkonzentrate mittels Direktdampfinjektion verwendet. Die quantitative Trennung der Fraktionen wurde mittels Partikelgrößenanalyse überprüft und die Größenänderung der Caseinmicellen über die Erhitzungsintensität für unterschiedliche Trockenmassen untersucht. Die gewonnen Fraktionen wurden mittels einer weiterentwickelten RP-HPLC Methode auf die relativen Gehalte an einzelnen Caseinen und Molkenproteine analysiert. Daraus sollten Erkenntnis-

se zum Mechanismus der Koagulation und deren Verlauf erhalten werden. Die Untersuchungen zeigten, dass die Vergrößerung des hydrodynamischen Radius der Caseinmicellen ebenso wie die Neigung zur hitzeinduzierten Koagulation mit steigender Trockenmasse der Konzentrate und der Erhöhung der Erhitzungstemperatur zunahm. Koaguliertes Casein bestand vorwiegend aus calciumsensitiven α_S- und β-Casein. Mit steigender Erhitzungsintensität stieg der Anteil an dissoziiertem Casein, wobei vor allem κ-Casein dissoziierte. Ab einem dissoziierten Anteil von 30-35% des κ-Caseins setzte die Koagulation der Caseinmicellen ein. Dabei hatte sich jedoch auch die mittlere Größe der Micellen verdoppelt, sodass mehrere Destabilisierungsmechanismen der Micellen wahrscheinlich sind, die zur Koagulation führen.

Die Erhitzungsversuche von Magermilchkonzentraten und die daraus gewonnenen Erkenntnisse zum Zusammenhang zwischen der Trockenmasse (TM) der Konzentrate und den kritischen Temperatur-Zeit-Bedingungen der Erhitzung sollten noch bis zur Ableitung einer Kinetik der hitze-induzierten Aggregation erweitert werden. Dazu wurden dünnwandige mit Magermilchkonzentrat unterschiedlicher Trockenmasse (12-33% TM) befüllte Edelstahlröhrchen mit Sattdampf auf Temperaturen zwischen 103 und 131 °C zwischen 6 und 5000 s erhitzt und jeweils der koagulierte Anteil an Casein durch Zentrifugation quantitativ abgetrennt. Der Koagulationsverlauf des Konzentrates mit 27% TM, das bei unterschiedlichen Temperaturen erhitzt wurde, sowie der unterschiedlichen Trockenmassen, die bei 116 °C erhitzt wurden, ließ sich zeitabhängig durch ein Weibull-Modell beschreiben. So konnten kritische Grenzen für die Erhitzbarkeit von Magermilchkonzentraten mit unterschiedlichen Trockenmassen, die Temperaturabhängigkeit der Reaktionsgeschwindigkeit abgeleitet werden. Eine Berechnungsgrundlage für nicht-isotherme Erhitzung der Konzentrate durch stetige Integration der Temperatur-Zeit-Effekte wurde erstellt. Die Validierung des Modells erfolgte anhand der Daten zur Hitzeaggregation von Magermilchkonzentraten unter den Bedingungen der Direkterhitzung bei höheren Temperaturen und deutlich kürzeren Heißhaltezeiten.

Ein Vergleich der Ergebnisse der Hitzestabilitätstest im Labormaßstab mit den erhaltenen kritischen Temperatur-Zeit-Kombinationen von Magermilchkonzentraten der Direkterhitzung im Technikumsmaßstab sowie den reaktionskinetischen Berechnungen ermöglicht, die Hitzestabilität von beliebigen Milchkonzentraten im Labormaßstab zu ermitteln und eine Vorhersage bezüglich der Hitzestabilität unter kontinuierlicher Erhitzung zu treffen.

Weitere Erkenntnisse zum Mechanismus der Hitzekoagulation und dem Zusammenhang der hitzeinduzierten Dissoziation von κ-Casein und der Micellvergrößerung mit der Aggregation der Caseinmicellen erschienen in dem komplexen Gemisch Magermilchkonzentrat schwierig. Dies sollte daher mithilfe eines Modellsystems, eines micellaren Caseins, das durch Aufreinigung mittels Diafiltration gewonnen wurde, untersucht werden. Diese molkenprotein- und laktosefreie Caseinmicellsuspension wurde durch mehrfache Diafiltration mit simuliertem Milchultrafiltrat (SMUF), einer synthetischen Salzlösung, die dem natürlichen Milchserum sehr nahe kommt, hergestellt. Das SMUF wurde basierend auf den Ergebnissen der dafür etab-

lierten ionenchromatographischen Trennung der Milchsalze im Milchserum entwickelt. Dieses SMUF wurde bezüglich seiner Salzzusammensetzung und der chemisch-physikalischen Eigenschaften in Abhängigkeit der Temperatur und des pH-Wertes eingehend charakterisiert. Die Zielstellung einer identischen Zusammensetzung und der Gleichheit der wesentlichen chemisch-physikalischen Eigenschaften des SMUF konnte insbesondere für unterschiedliche Arbeitstemperaturen ohne die Kristallisation von Calciumphosphat erreicht werden. Die Calciumaktivität in Abhängigkeit der Zusammensetzung des SMUF und des pH-Wertes ließ sich durch Messungen mit einer calciumselektiven Elektrode nachweisen. Der Bereich der Übersättigung konnte durch den Vergleich mit der pH-abhängigen Calciumaktivität von Magermilch bestimmt werden.

Mithilfe dieses mit SMUF diafiltrierten Casein-Modellsystems war eine gezielte Variation einzelner Millieufaktoren möglich. Die Interferenz der Maillard-Reaktion mit der analytischen Bestimmung der Caseine mittels RP-HPLC und dem Mechanismus der Koagulation wurde minimiert. Die verstärkte Dissoziation von κ-Casein infolge des Thiol-Disulfid-Austausches mit β-Lactoglobulin wurde ebenso unterbunden. Die Untersuchung des Einflusses des pH-Wertes auf die hitzeinduzierte Koagulation von micellarem Casein ergab, dass bei pH > 6,7 die Koagulation sehr stark limitiert war, aber eine Dissoziation eines großen Anteils der Caseine, insbesondere von κ-Casein, beobachtbar war. Mit steigender Temperatur erhöhte sich der Anteil an dissoziiertem Casein bei gleicher Heißhaltezeit. Mit sinkendem pH-Wert ergab sich eine stetige Verringerung des dissoziierten Caseins über die Heißhaltezeit bei 116 °C. Jedoch ergab sich bei niedrigen pH-Werten eine deutliche Vergrößerung der Caseinmicellen, die bei der Zugabe von löslichem Calcium auch beobachtet werden konnte. Die zusätzliche Erhöhung der Ionenstärke nach Calciumzugabe und Verringerung des pH-Wertes induzierte schließlich die Koagulation der Caseinmicellen zu diskreten Partikeln. Dies lässt den Schluss zu, dass im pH-Wert-Bereich von 6,3-7,2 mehrere die Caseinmicelle destabilisierende Faktoren wie ein hoher Gehalt an löslichem Calcium, eine erhöhte Ionenstärke und eine bestimmte Erhitzungsintensität erforderlich sind, um die kolloidale Stabilität der Caseinmicellen so zu schwächen, dass die Aggregation auftritt. Die hitzeinduzierte Aggregation tritt dann ein, wenn all diese Faktoren kombiniert vorliegen, so wie es in Konzentraten aus Mager- und Vollmilch der Fall ist. Niedrige pH-Werte und hohe Calciumgehalte führen dabei insbesondere zu einer Lockerung der inneren Struktur der Caseinmicelle, sodass die neugeschaffene calciumsensitive Oberfläche der Micellen durch die Exposition calciumempfindlicher Caseine die Aggregation begünstigt. Die Dissoziation von κ-Casein erscheint nicht im unmittelbaren Zusammenhang mit der Koagulation, insbesondere bei pH < 6,7, sollte jedoch als eine Abweichung von der originären Micellstruktur der Caseine gesehen werden, die die physikalischen Eigenschaften der Micellen verändert.

Eine Erweiterung des Begriffs der ‚Hitzestabilität' der Caseinmicelle erscheint notwendig, sodass sowohl die Dissoziation von Caseinen als auch die Schwächung

der inneren Struktur, die mit der Koagulation in Zusammenhang steht, berücksichtigt werden.

Die Hauptergebnisse dieser Arbeit lassen sich wie folgt zusammenfassen.

- Eine reaktionskinetische Beschreibung der hitze-induzierten Koagulation von Magermilchkozentraten im Labor- und Technikumsmaßstab zur Bestimmung und Berechnung kritischer Temperatur-Zeit-Bedingungen ohne erkennbare Koagulation der Konzentrate.
- Studien zur Dissoziation und Koagulation einzelner Caseine in konzentrierter Magermilch, die mittels direkter Dampfinjektion erhitzt wurde.
- Die Entwicklung eines simulierten Milchultrafiltrates, z.B. geeignet für die Aufreinigung von Caseinmicellen, um gezielte Veränderungen in der Serumzusammensetzung in Bezug auf hitzeinduzierte Veränderungen an Caseinmicellen zu untersuchen.
- Untersuchungen zu hitze-induzierten Veränderungen der Caseinmicellstruktur und der Dissoziation von Caseinen in erhitztem micellarem Casein in Abhängigkeit der Ionenstärke, pH-Wert und löslichem Calcium.