The Impact of Milk Properties and Process Conditions on Consistency of Rennet-coagulated Curd and Syneresis of Rennet Curd Grains

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SYMBOLS

a	time constant [min]
$d_{3,2}$	volume-to-surface mean diameter [µm]
D_B	deformation at fracture [%]
E_A	activation energy [J/mol]
F-60-value	curd firmness after 60 min of coagulation [N]
F_B	fracturability [N]
F_D	force at 33 % of sample deformation [N]
F_{cm}	fat content of the cheese milk [%]
F_{rm}	fat content of the standard milk [%]
G'	storage modulus [Pa]
G''	loss modulus [Pa]
G'_{∞}	storage modulus after infinite time [Pa]
i	concentration factor of microfiltration [-]
$k_{T_{ref}}$	rate constant at reference temperature [s]
k	temperature dependent rate constant [1/s]
M_a	actual cheese moisture content [%]
M_r	cheese moisture content of the standard [%]
m_{Milk}	weight of milk used for cheese manufacture [kg]
$m_{Permeate}$	weight of permeate released during microfiltration [kg]
$m_{Retentate}$	weight of retentate used for cheese manufacture [kg]
m_t	mass of curd grains after time t [g]
m_{Water}	the amount of water to be added after whey drainage [kg]
$m_{Whey\ to\ Drain}$	weight of whey to be drained prior to curd washing [kg]
m_0	initial mass of curd grains at time t = 0 [g]
p_1	homogenization pressure in the first stage [MPa]
n	order of reaction
r^2	coefficient of correlation
r^2_{nl}	non-linear coefficient of correlation
P_{cm}	protein content of the cheese milk [%]
P_{rm}	protein content of the standard milk [%]
T	absolute temperature [K]
T_{ref}	reference temperature [K]
t	time
$t_{1/2}$	time to reach half of DM_{max}, RWR_{max} [min]
$t(DM_{45min})$	time in min to reach the equivalent dry matter found in standard milk curd grains after 45 min of syneresis
Y_a	actual cheese yield [%]
Y_a*	adjusted actual cheese yield [%]

Y_{afpam}	yield per 100 kg of milk normalized to reference fat and protein levels [%]
Y_i	yellow index
Y_{ma}	moisture-adjusted yield [%]
Y_{mafpam}	moisture-adjusted yield per 100 kg of milk normalized to reference fat and protein levels [%]

GREEK SYMBOLS

δ	phase angle [°]
ϑ	temperature [°C]
τ	characteristic constant in s

CONSTANTS

R	universal gas constant [8.314 J/(mol·K)]

ABBREVIATIONS

ad RC	adjusted recovery
ANOVA	analysis of variance
AR	aggregation rate
BC	before Christ
$CaCl_2$	calcium chloride
CF	curd firmness
CFR	curd firming rate
cfu	colony forming units
CI	circle index
CLSM	confocal laser scanning microscopy
CT	cutting time
CTT	curd treatment time
DC	Dahlem Cashmere
DM	dry matter
DM_0	dry matter of a curd grain at the beginning of syneresis
DM_t	dry matter of a curd grain at time t
DM_W	dry matter of effluent whey
DM_{max}	dry matter of curd grains after infinite curd treatment time
D.M.S.	dynamic model system
EPS	exopolysaccharide
eq.	equation

FDM	fat-in-dry-matter
FM_{EPS}	fermented medium
FM_{EPS}th	thermisation of the fermented medium
GF	German Fawn
GP	gradient of permeability
GRAS	generally recognized as safe
GW	German White
La	lactalbumin
LAB	lactic acid bacteria
Lac.	*Lactococcus*
Lb.	*Lactobacillus*
Lg	lactoglobulin
LOF	lack of fit
MF	microfiltration
MNFS	moisture in non-fat solids
NaOH	sodium hydroxide
n.d.	not determined
n.h.	not homogenized
NS	not significant
PIDS	Polarisation Intensity Differential Scattering
RC	recovery
RCT	rennet coagulation time
rpm	revolutions per minute
RSM	response surface methodology
RT	ripening time
RWR	relative whey release/removal
RWR_{max}	relative whey release/removal of curd grains after infinite time
RWR_{60min}	relative whey release/removal of curd grains after 60 min
RWR_{240}	relative whey release/removal of curd grains after 240 min
S.	*Streptococcus*
s.d.	standard deviation
s.e.	standard error
subsp.	subspecies
S-to-D	starter-to-drain time
th	thermisation
TMP	transmembrane pressure
UF	ultrafiltration
v	volume
w	weight
WP	whey protein

1 INTRODUCTION

1.1 Scope

In cheese manufacture, syneresis is a phenomenon that occurs spontaneously after cutting rennet gel into grains. It causes the release of whey from the casein-based network of the grains and their dry matter increases. Syneresis is the key step in cheesemaking and it strongly influences cheese yield efficiency and likewise quality, since the degree of whey release determines the moisture content of the raw cheese by which rheological and sensory characteristics of the cheese are affected. Since cheesemaking is a complex process influenced by a multitude of factors, any intervention in the cheesemaking procedure, i.e. in cheese milk composition, microbial fermentation and applied technology not only affects syneresis but likewise gel formation and gel consistency at cutting. The respective gel consistency at cutting strongly influences the efficiency of cheesemaking. Insufficient firmness of the gel while cutting leads to casein grains with a weak structure stability and provokes losses of fat and protein. However, a firm and compact structure of grains in consequence of a too long coagulation time leads to an unnecessary delay of the total process and additionally impairs syneresis. Modern cheesemaking relies more and more on the implementation of innovative technology, e.g. microfiltration (MF), or on the addition of tailor-made starter bacteria and hydrocolloids to remain competitive in the production of commodity-type cheeses. In order to gain better understanding of the interaction of the various cheese processing steps and factors like the addition of exopolysaccharide (EPS)-producing lactic acid bacteria or the application of technologies like homogenization and MF, the work dealt with the following objectives:

- Implementation of EPS-producing cultures in the manufacture of soft cheese
- Study of the three-dimensional syneresis of rennet curd grains under defined conditions in order to propose a kinetic model for predicting syneresis
- Development of strategies to evaluate cheesemaking properties of milk from different breeds and species of ruminant
- Analysis of the interrelated effects of homogenization, MF and pH on rheological properties of rennet-induced milk gels, curd grain consistency and syneresis

- Evaluation of the potential of the combined application of homogenization and microfiltration in semi-hard cheese manufacture and study of its feasibility

1.2 Outline

Chapter 2 “Background” gives a brief overview of cheese in general, rennet-induced gel formation and syneresis.

Chapter 3 “Exopolysaccharide-producing lactic acid bacteria in the manufacture of soft cheese” demonstrates the effects of exopolysaccharide (EPS) and EPS-producing strains on syneresis, acidification, soft cheese composition and quality. A strategy is presented to overcome problems of ripening by adapting the manufacturing parameters. The impact of temperature, time and thermisation of the fermented medium on acidification and syneresis of the curd grains is discussed.

In **Chapter 4** “Comparison of models for the kinetics of syneresis of curd grains made from goat’s milk” a kinetic model is proposed for approximating syneresis of rennet curd grains depending on milieu conditions and curd grain size.

Chapter 5 “Strategy to evaluate cheesemaking properties of milk from different goat breeds” describes the influence of milk composition and origin on coagulation properties of rennet-induced milk gels, rennet curd consistency after 60 min of coagulation and syneresis. It demonstrates that following this protocol cheesemaking potential of a certain milk is assessable.

Chapter 6 “Impact of homogenization and microfiltration on rennet-induced gel formation” deals with the relationship between processing parameters, namely homogenization pressure, concentration factor of microfiltration and pH, and rheological properties of rennet-induced milk gels.

In **Chapter 7** “Effect of homogenization, microfiltration and pH on curd firmness and syneresis of curd grains” the kinetic model presented in Chapter 4 is adapted to the syneresis of rennet curd grains made from concentrated milk. Hence, the chapter is focused on the interrelated effects of homogenization pressure, concentration factor of microfiltration and pH on curd firmness and syneresis of rennet curd grains.

Chapter 8 “Feasibility study for semi-hard cheese manufacture: Yield and functionality of full-fat semi-hard cheese as influenced by the combination of microfiltration and homogenization” illuminates the potential of implementing homogenization and microfiltration in semi-hard cheese manufacture. Along with chemical analyses of the milk, component recovery, cheese yield and cheese functionality are examined to highlight the influence of milk processing. Furthermore, a comparison is made between two procedures to demonstrate that a cheesemaking procedure usually applied can be adapted and simplified, if appropriate preliminary experiments are carried out.

2 BACKGROUND

2.1 Cheese

Cheese is the generic name for a group of fermented milk-based products, manufactured in a wide range of flavours and forms all over the world. During cheesemaking the larger part of the nutritious of milk is concentrated and the primary objective is to preserve the constituents of milk. However, cheese has evolved to become a food of haute cuisine with epicurean qualities as well as being highly nutritious (Fox and McSweeney 2004).

It is believed that the origin of cheese is located in a region known as the “Fertile Crescent”, i.e. besides the Tigris and Euphrates rivers what is now southern Turkey and the Mediterranean coast, and evolved some 8000 years ago (Kammerlehner 2003; Fox and McSweeney 2004). Apparently, goats and sheep were the first dairy animals domesticated, but cattle have become the dominant dairy species in most parts of the world. For instance, the sheep and goat milk production in the European Union was in 2002 only 4 % of the cow milk production (Dubeuf and Le Jaouen 2004). The first cheese, presumably a sort of fresh cheese, was produced by accident and by a combination of events - the ability of a group of lactic acid bacteria (LAB) to grow in milk and to produce enough acid to reach the isoelectric point of the caseins, at which these proteins coagulate. Soon, it was realized that breaking or cutting the gel causes separation into curd grains and whey and that the shelf-life of the grains could be extended by dehydration and/or adding salt. Furthermore, the by-product acid whey was recognized as a pleasant, refreshing drink and has been considered to have medicinal benefits.

While lactic acid is believed to be the original milk coagulant, an alternative mechanism was also recognized from an early date, proteolytic enzymes. Enzymes capable of modifying the casein system in milk are widespread in nature, e.g. bacteria, moulds, plant (fig and thistle) and animal tissue, but an obvious source is the animal stomach (Fox and McSweeney 2004). In all likelihood, people discovered this source by storing milk in bags made from animal skins that was a common custom before the development of pottery (about 5000 BC). Milk extracted enzymes (chymosin and some pepsin) from the stomach tissue lead to its coagulation during storage.

A great diversity of cheeses is produced and the composition and properties of the final cheese are determined by the characteristics of the raw material (usually bovine, ovine, caprine or buffalo milks, LAB, coagulant and NaCl) and the processing conditions. Kammerlehner (2003) suggested that there are more than 2000 varieties of cheese existing all over the world and a list of 1400 varieties is presented by the Wisconsin Center of Dairy Research (www.cdr.wisc.edu/applications/specialty_cheese/cheese_database.html). The most common criterion for the classification of cheese is texture which is related mainly to the moisture content of cheese. According to § 6 (Käseverordnung 1986), cheese is grouped by their content of moisture in non-fat solids (MNFS) in hard (MNFS < 56 %), semi-hard (MNFS > 54 - 63 %), soft (MNFS > 67 %) and fresh cheese (MNFS > 73%).

World cheese production in 2005 was 17.8 million tonnes. Germany was the second largest cheese producer worldwide with approximately 1.8 million tonnes (Rasmussen 2006). The German cheese consumption per capita increased from 19 kg in 1995 to 22 kg in 2004 (Rasmussen 2006) demonstrating the outstanding economic potential of cheese production for the dairy industry. A comparison of cheese production by types indicates that this increase may be mainly attributed to semi-hard/hard and fresh cheese production since it increased from 1995 to 2004 by 19 % and 29 %, respectively.

Cheeses are biologically and biochemically dynamic systems and are therefore inherently unstable. Throughout manufacture and ripening, cheese production represents a series of consecutive and concomitant biochemical and technical processes which, if balanced, lead to products with desirable aroma, flavour and texture, but if unbalanced, result in off-flavours and impaired texture. Hence, the study of cheese manufacture and ripening is required to control and optimize the cheesemaking procedure; in particular modern cheese production relies on the application of natural science and engineering, comprising the use of industrial enzymes, complex fermentations, sophisticated engineering and a dynamic biochemistry during ripening. Therefore, this study particularly concerns rennet-type gels to generate a better understanding of underlying principles regarding gel formation, curd consistency and syneresis as affected by a number of cheesemaking parameters.

2.2 Rennet-induced Gel Formation

The essential step in the manufacture of nearly all cheese varieties is the conversion of liquid milk to cheese curd. By adding rennet or other milk-clotting enzymes to milk the casein fractions of milk, which constitute about 80 % of the total milk protein (Walstra and Jenness 1984), form a gel that entraps fat, if present.

The caseins are a family of phosphoproteins and in bovine milk it consists of four main characteristic gene products, designated α_{s1}-, α_{s2}-, β- and κ-caseins. Two post-translational modifications of the proteins have a major impact on the physico-chemical, functional and assembly properties of the proteins. These reactions are glycosylation and phosphorylation (Horne and Banks 2004). Only κ-casein is found glycosylated in the hydrophilic C-terminal end of the κ-casein molecule carrying relatively short sugar chains. All the caseins are phosphorylated to varying extents, whereas κ-casein is unique among the caseins in containing only one phosphoseryl residue.

In uncooled milk (pH 6.7) almost all casein fractions are incorporated in aggregates of colloidal size with a diameter between 20 and 300 nm and a molecular weight of 10^8, the casein micelles (Bijgaart 1988). These aggregates contain a high proportion of the available calcium and inorganic phosphate and are highly hydrated structures with typical hydration values of 2 - 4 g water per g protein (Horne and Banks 2004). Controversy still exists about the micelle structure of bovine casein micelles among researchers. A variety of models have been proposed to describe the structure of bovine casein micelles and these models have generally fallen into three categories: coat-core models, internal structure models, and subunit models (McMahon and McManus 1998). Recently, Horne (2002) suggested to treat the caseins as block copolymers which explains self-association, adsorption and micellar assembly of the casein fractions. Without going into detail, the electron micrograph published by Dalgleish *et al.* (2004) in Figure 2.1 can serve to illustrate the micellar structure of casein in milk.

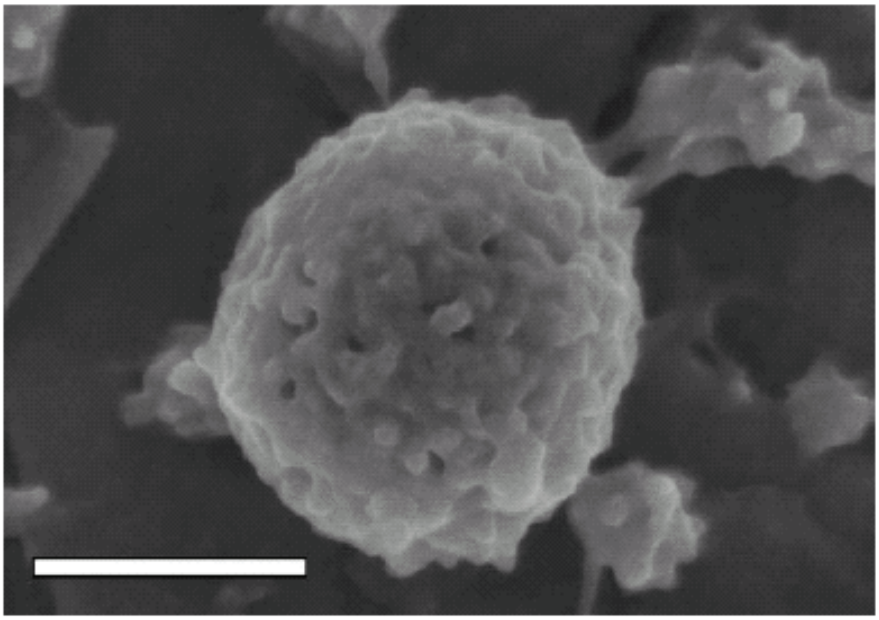

Figure 2.1: Electron micrograph of an individual casein micelle, made using the technique of field-emission scanning electron microscopy (Dalgleish *et al.* 2004). Samples were prepared on a carbon substrate (flat or foliated background). No coating techniques were employed. Note the apparent connection between the main micelle and a subsidiary structure, which may be a part of the micelle being dissociated. Scale bar = 200 nm.

The micelles are hold in suspension by repulsive forces until reduced by some external influence like rennet treatment resulting in a changed zeta potential. The colloidal stability of the micelle arises from the presence of a sterically stabilizing outer layer of κ-casein molecules, the C-terminal end of which extends out into the solution (Walstra 1990; Creamer *et al.* 1998). The role of chymosin and respectively rennet is to proteolyze κ-casein, splitting it at the Phe105-Met106 bond, so that the subsequently exposed micelle cores start to aggregate (Horne and Banks 2004). Thus, the renneting of milk is the result of at least two processes, the attack on κ-casein by chymosin and the flocculation of the destabilized micelles, whereas the latter process only becomes visual in untreated milk when about 80 % of the κ-casein has been hydrolyzed (Dalgleish 1979). It is to be mentioned that proteolysis is certainly not complete before the aggregation starts. The aggregation can be described by Smoluchovski kinetics and relies on van der Waals attraction, specific ion pair formation and hydrophobic effects.

When flocculation and aggregation proceed undisturbed, a continuous network is formed. Generally fat globules are trapped in the pores and thus act as a non-reactive filler (Bijgaart 1988). The contact region between the casein particles

changes from “touching” to “fusion” during ongoing gelation and thickening of strands appear so that after some hours the original particles making up the gel can no longer be distinguished. During this process the contribution of the various types of bonds may change and recently, the dominating forces stabilizing rennet curd were attributed to calcium bonds (Keim 2005). The increase of the dynamic moduli, in particular the storage modulus G’, during several hours after rennet addition is found in rheological measurements (Dijk 1982; Roefs 1986; Guinee *et al.* 1994; Auldist *et al.* 2001) and depends on the number, the strength and the relaxation behaviour of the bonds (Zoon *et al.* 1988). Due to its sensitivity, rheometry was therefore widely used to follow and to characterize rennet-induced gel formation.

2.3 Syneresis of Rennet-induced Milk Gels

Cheese manufacture is in principal a dehydration process in which fat and casein of milk are concentrated up to 12-fold (Fox and McSweeney 2004) and the basic key step initiating this process is syneresis. Consequently, it is useful to understand and quantitatively describe syneresis as a function of milk properties and process conditions. This is in particular essential when new methods or process steps are introduced and according to Dejmek and Walstra (2004) this concerns the following aspects:

- regulation of the moisture content of the cheese implies controlling syneresis;
- rate of syneresis affects the method of processing, and thereby the equipment and time needed, and the losses of fat and protein in the whey;
- rate of syneresis in relation to other changes (e.g. acidification, proteolysis, inactivation of rennet enzymes) affects cheese composition and properties;
- the way in which syneresis of curd grains proceeds may affect the propensity of the grains to fuse into a continuous mass during shaping and/or pressing;
- differences in syneresis throughout a mass of curd cause differences in cheese composition between loaves of one batch and between sites in one loaf;
- after the cheese loaf has been formed, it may still show syneresis and hence loss of moisture.

A distinction has to be made between endogenous syneresis (i.e., syneresis due to concentration of the gel under absence of external forces, such as gravity) and syneresis due to external pressure (Vliet *et al.* 1991). The main driving force of endogenous syneresis is attributed to the rearrangement of para-casein particles in the network by Brownian motion and deformation of the strands (Dijk 1982; Walstra *et al.* 1985). According to Bijgaart (1988) this rearrangement can occur because para-casein micelles probably are reactive over their entire surface and by far, the greater part of the surface of each particle does not touch or form bonds with another one. These non-aggregated particles which attach to the existing network may promote the rearrangement in the initial stages after gel formation by becoming an extended target point for dangling or moving strands. This leads to a more compact network with an increase in the number of bonds and hence decreases the total free energy (Walstra and Dejmek 2004). In later stages, breaking of some of the strands will be needed to attain the more compact configuration because the para-casein particles are almost immobilized in the network. Therefore, the network has to be locally deformed to form new junctions which results in the formation of more and less dense regions elsewhere, increasing the permeability of the constrained gel and has been designated microsyneresis. This process is illustrated and discussed in detail by Dijk (1982). Spontaneous breakage of strands and building of new cross-links is possible if (1) the bonds in a strand can relax and (2) the number of bonds in a strand is not too high, say locally only one particle thick and the junction zones are fairly small (Vliet *et al.* 1991).

Values of the initial endogenous syneresis pressure were found to be between 1 and 3 Pa and due to this very small pressure it takes 7 hours at 30 °C for a slab of 6 mm thickness to be reduced to 3 mm (Dijk 1982). Hence, external or mechanical pressure is essential to allow cheesemaking under realistic conditions. The large effect of external pressure on syneresis, for instance caused by cutting and stirring the curd, temperature and pH, is in detail reviewed by Walstra *et al.* (1985) and Walstra and Dejmek (2004) as well as the pros and cons of various methods applied to follow syneresis. Comprehensive studies were carried out concerning one-dimensional syneresis, i.e. horizontal slabs of renneted milk were moistened at the top and thereafter, whey only could flow out at the top (Dijk 1982; Bijgaart 1988; Grundelius *et al.* 2000; Lodaite *et al*. 2000). Syneresis was followed by measuring the change in height of the slab, e.g. using laser technology. In this study, three-

dimensional syneresis as affected by a number of parameters was followed and modelled after cutting the curd into rennet curd grains with defined diameters.

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3 EXOPOLYSACCHARIDE-PRODUCING LACTIC ACID BACTERIA IN THE MANUFACTURE OF SOFT CHEESE

Abstract

Soft cheese was manufactured with exopolysaccharide (EPS)-producing strains of Lactococcus lactis subsp. cremoris (Lac. cremoris) and Lactobacillus sakei (Lb. sakei). EPS-producing strains were fermented in a growth promoting medium and added to the cheese milk in concentrations of 5 and 10 %. The effects of EPS and EPS-producing strains on cheese production and composition were investigated and compared to standard cheeses inoculated only with Streptococcus thermophilus (S. thermophilus). Compared to the standard, syneresis during cheese processing was delayed. Due to a high moisture content of EPS-containing cheese and low pH values, ripened cheeses showed a soft, bitter outer layer, a chalky, acidic core and dead mould. The process was improved by an appropriate acidification and whey removal during cheese manufacture. Ad hoc, acidification and whey removal were studied individually in model experiments in order to demonstrate their time and temperature dependency. Additionally, the influence of thermisation of the fermented medium on acidification and syneresis was studied. The results for acidification and syneresis were combined and target values for curd grain treatment were determined. By adjusting the manufacturing parameters, production of well tasting soft cheeses with strains of Lac. cremoris and Lb. sakei using 10 % fermented, thermised medium was possible.

Keywords: *Exopolysaccharide; Soft Cheese; Lactic Acid Bacteria*

Thomann, S., Bonte, K. and Hinrichs, J. (2006). Exopolysaccharide-producing lactic acid bacteria in the manufacture of soft cheese. Milchwissenschaft - Milk Science International *61*, 165-169.

3.1 Introduction

Exopolysaccharides (EPS) are produced by a great variety of bacteria, including lactic acid bacteria such as *Leuconostoc mesenteroides*, *Streptococcus mutans*, *Streptococcus thermophilus* (*S. thermophilus*), *Lactococcus lactis* and dairy *Lactobacillus* spp. (Lb.). Most lactic acid bacteria are food grade organisms with generally recognized as safe (GRAS) applications, so that the use of their EPS in food has an obvious advantage over polysaccharides built by non-food grade bacteria, such as dextrans, gellan, pullulan, xanthan and bacterial alginates (Higashimura *et al.* 2000). EPS are excreted into the growth medium as slime or remain attached to the bacterial cell wall, thus forming capsular EPS (Cerning 1990).

EPS-producing strains can reduce syneresis and enhance product texture and viscosity, so that these types of cultures are commonly used as a substitute for commercial stabilizers in yogurt manufacture (Cerning 1995; Hassan *et al.* 2003). They may also be a potential alternative for thickening agents to increase moisture content and improve texture attributes of reduced fat cheese. Kojic *et al.* (1992) isolated an EPS-producing strain, *Lb. casei* CG11, from soft, white, homemade cheese, indicating that EPS-producing strains are often part of the natural cheese flora.

In the cheese industry, application of EPS-producing starters has been extensively evaluated in low-fat Mozzarella cheese (Dabour *et al.* 2005). Several studies revealed that using EPS-producing strains of *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* increased the moisture content in low-fat Mozzarella cheese of about 1.7 to 4 % (Perry *et al.* 1997; Low *et al.* 1998; Perry *et al.* 1998; Bhaskaracharya and Shah 2001). Furthermore, texture and functional properties of the cheese were improved (Perry *et al.* 1997; Petersen *et al.* 2000; Broadbent *et al.* 2001). Recent studies of Dabour *et al.* (2005) showed that application of *Lactococcus lactis* subsp. *cremoris* (*Lac. cremoris*) increased the moisture content of Cheddar cheese of about 3.6 to 4.8 %, resulting in 0.29 to 1.19 % higher yield than cheese without EPS-producing culture. This increase is related to the water binding capacity of microbial EPS, which retards whey expulsion (de Vuyst and Degeest 1999).

The aim of this study was to cast light into the implementation of EPS-producing cultures in the manufacture of soft cheese.

3.2 Materials and Methods

Raw milk was provided by the research station Meiereihof (University of Hohenheim, Germany). The milk for soft cheese manufacture and further experiments was separated, pasteurized (at 74 °C for 30 s) and adjusted to a mean fat content of (3.42 ± 0.12)%. The average composition of the milk was (3.32 ± 0.14)% for protein and (12.13 ± 0.34)% for dry matter content.

Non-EPS-producing *Streptococcus thermophilus* TS-H 100 (*S. thermophilus*) and EPS-producing strains of *Lactococcus lactis* subsp. *cremoris* 322 (*Lac. cremoris*) and *Lactobacillus sakei* (*Lb. sakei*) were obtained from Danisco, Niebüll, Germany. VIS-START®10 medium (Danisco, Niebüll, Germany) was inoculated with 1 % of active culture. VIS-START®10 medium for *Lb. sakei* contained an addition of 10 % sucrose (D(+)-Sucrose, 4621.1, Carl Roth GmbH & Co, Karlsruhe, Germany). *Lac. cremoris* and *Lb. sakei* were incubated at 30 °C for 16 h and 48 h, respectively. In the following, these fermented media are referred to as FM_{EPS}.

Soft cheeses with a weight of approximately 150 g were manufactured from 4-8 kg of the pasteurized milk at 37 °C by the addition of 5 and 10 % (w/w) FM_{EPS} at the beginning of the preripening step and in the case of *Lb. sakei* at the end of the preripening step. Standard cheese was manufactured by adding 5 and 10 % (w/w) skimmed milk instead of FM_{EPS}. In some treatments, thermisation of the FM_{EPS} was carried out at 65 °C for 20 s (Kessler 2002) before the addition, indicated in the following as FM_{EPS}th. Thermisation of FM_{EPS} was introduced to decrease the amount of active lactic acid bacteria in order to reduce the influence of the added EPS-bacteria during cheesemaking. All batches were inoculated with non-EPS-producing *S. thermophilus* as starter culture in a concentration of 10 ml per 100 l milk and with *Penicillium candidum* NR (Danisco, Niebüll, Germany). Calcium chloride (calcium chloride dihydrate, 1.02382, Merck, Darmstadt, Germany) was added in a concentration of 0.01 % (w/w) and curd setting was induced by the addition of 0.02 % (v/w) chymosin (strength 1:15000, Chymosin ≥ 80 %, IP Ingredients GmbH, Süderlugum, Germany).

Syneresis experiments were carried out at 37 °C with the Dynamic Model System previously described by Huber *et al.* (2001), simulating the whey release of curd grains during soft cheese manufacture. Different from their method, curd grains had

an edge length of 22 mm and only one of the cut grains was transferred in 25 ml of tempered sweet whey. Sweet whey with a dry matter of 5.2 % and pH 6.3 was reconstituted from spray-dried sweet whey powder (Schwarzwaldmilch, Offenburg, Germany). Syneresis was determined after 5, 10, 20, 30, 60, 90, 120 and 180 min and expressed as relative whey removal (RWR). All trials were renneted after a preripening time of 60 min at 37 °C. Cutting time was calculated individually by multiplying the rennet coagulation time by factor 4. Milk and FM_{EPS} for the experiments as well as for the fermentation experiments were treated and adjusted as described afore for cheese manufacture.

In addition, fermentation experiments were carried out over a period of approximately 24 h (pH 522, Schott, Mainz, Germany). According to the temperature profile during soft cheese manufacture, fermentation was started at 37 °C, once pH reached 6.2 (usually pH at moulding and forming the cheese), the fermentation temperature was decreased to ambient temperature at around 20 °C. Below pH 5.1, samples were cooled over night to 12 °C.

The composition of milk and cheese was determined by means of standard methods (VDLUFA 2003). The protein content was measured by means of the DUMAS method (FP-528, Leco Instrumente GmbH, Mönchengladbach, Germany).

3.3 Results and Discussion

Regarding Table 3.1, due to addition of 10 % FM_{EPS}, the pH value at the beginning of the preripening step was 6.32 for *Lac. cremoris* and 6.24 for *Lb. sakei*, and was thus lower than the standard (pH 6.56). Due to the addition of FM_{EPS} with a pH ranging between 4.49 and 4.19, the pH of the cheese milk at the beginning of cheese manufacture decreased. The difference in pH was maintained for cheese milk incubated with FM_{EPS} of *Lac. cremoris* during the whole cheesemaking process, whereas the pH of *Lb. sakei* approached the standard pH. According to the results in Table 3.1, it even seems that acidification after renneting is delayed for *Lb. sakei* compared to the standard and *Lac. cremoris*. Still, pH at renneting (6.14 and 6.27) was lower than standard and corresponds rather to the pH at moulding. The pH drop in the preripening step after the addition of FM_{EPS} is much higher compared to the standard, indicating that EPS-producing cultures are still active, transforming lactose

to lactic acid after addition. Because of the different pH values, cheese manufacture conditions were not comparable and resulted in changed process schedules. The dry matter of raw cheeses containing 10 % FM_{EPS} was lowest and highest for the standard (Table 3.1). Due to the increased moisture content of the EPS-containing cheese and the high amount of active culture and enzymes, cheese ripening was influenced and the cheeses showed a soft, bitter outer layer, a chalky, acidic core and dead mould.

Table 3.1: pH values of cheese milk and pH values and dry matter (DM) of raw cheese at the main process steps during cheese manufacture, supplemented with FM_{EPS}.

Strain	Addition of FM_{EPS} in %	$pH_{preripening}$	$pH_{renneting}$	$pH_{moulding}$	$pH_{chilling}$	$pH_{raw\ cheese}$	$DM_{raw\ cheese}$ in %
Standard	*	6.56	6.53	6.16	5.10	4.97	40.19
Lac. cremoris	10	6.32	6.19	6.02	4.88	4.69	33.86
	5	6.49	6.36	6.09	5.06	4.82	37.77
Lb. sakei	10	6.24	6.14	6.08	5.17	5.03	33.45
	5	6.39	6.27	6.13	5.09	5.00	38.75

*: addition of 10 % skim milk; DM: dry matter; FM_{EPS}: fermented medium

On the one hand, the cheesemaking experiments confirmed that soft cheese containing FM_{EPS} retained more serum resulting in high moisture contents. On the other hand, the high moisture content caused ripening problems. Thus, the processing had to be technologically adapted.

Therefore, controlled acidification and controlled whey removal is required. In order to improve the textural and sensory properties of the cheese, both processes were examined individually. Additionally, the influence of FM_{EPS} thermisation (th) on acidification and whey removal was studied. The heat treatment reduced the amount of colony forming units per ml (cfu/ml) from $5.52 \cdot 10^8$ cfu/ml to $2.04 \cdot 10^6$ cfu/ml for *Lb. sakei* and from $2.52 \cdot 10^8$ cfu/ml to $3.03 \cdot 10^4$ cfu/ml for *Lac. cremoris*.

Figure 3.1a demonstrates that thermisation of *Lac. cremoris* (FM_{EPS}th) resulted in a reduced acidification during fermentation compared to standard. Cheese milk with FM_{EPS}th was only slightly lowered in pH during the fermentation. Milk with FM_{EPS}th + standard showed a lapse of pH comparable to the standard, and the final pH of about 5 after 24 h was also comparable. In contrast, cheese milk supplemented only with FM_{EPS} was acidified too fast and to a final pH of about 4.4, indicating that *Lac. cremoris* was still active although samples were stored below

12 °C after 6 h of fermentation. *Lb. sakei* acidified cheese milk only slightly and together with starter culture (FM_{EPS} + standard), acidification was even delayed compared to the pH progression of the standard (Figure 3.1b). FM_{EPS}th + standard gave a pH progression that was closer to the standard than all other treatments. It has to be highlighted, that for both EPS-producing cultures at concentrations of 5 and 10 %, the combination of thermisation of FM_{EPS} and addition of starter culture (FM_{EPS}th + standard) gave fermentation curves comparable to the standard.

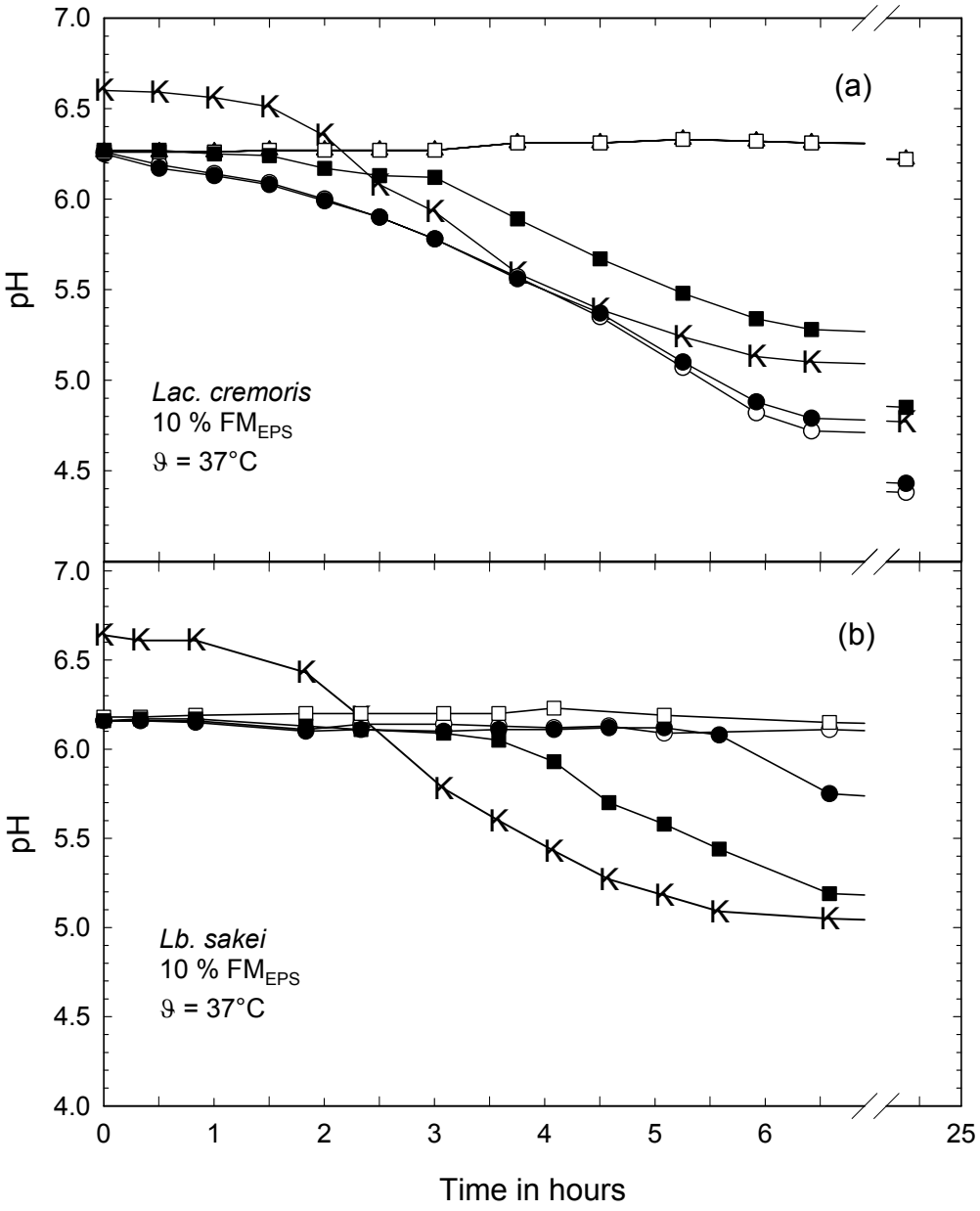

Figure 3.1: Simulated fermentation of cheese milk during cheesemaking with addition of 10 % fermented medium (FM_{EPS}) of (a) *Lac. cremoris* and (b) *Lb. sakei*, respectively, at 37 °C (K: standard; ○: FM_{EPS}; ●: FM_{EPS} + standard; □: FM_{EPS}th; ■: FM_{EPS}th + standard; th: thermisation). Below pH 6.2: fermentation at ambient temperature; below pH 5.1: fermentation at 12 °C.

In parallel, syneresis experiments were conducted with cheese milk supplemented with 5 and 10 % FM_{EPS}th and FM_{EPS}, respectively, together with starter culture. In Figure 3.2, the results of both syneresis and fermentation experiments are combined for *Lac. cremoris*. Soft cheeses are usually moulded at a pH between 6.1 and 6.3, and at a relative whey removal (RWR) of about 50 %. The latter target value is based on data of Ramet (2000), who discussed a dry matter content of the curd at moulding time typically being about 22 % in the manufacture of soft cheese. As a consequence of different cutting times, syneresis curves had different starting points for each trial (see Chapter 3.2). As an example, syneresis and fermentation curves of 10 % FM_{EPS}th in Figure 3.2 shall be taken to illustrate how curd treatment time was determined by means of the target values. Renneting was carried out after 60 min. The gelling point was determined after 8.2 min, so that multiplication with factor 4 gave a coagulation time of 33 min. Consequently, the gel was cut after 93 min, inducing syneresis. After approximately 165 min, RWR was almost 50 % at a pH in between 6.1 and 6.2. Therefore, both target values were met at a curd treatment time of 72 min (165 min - 93 min).

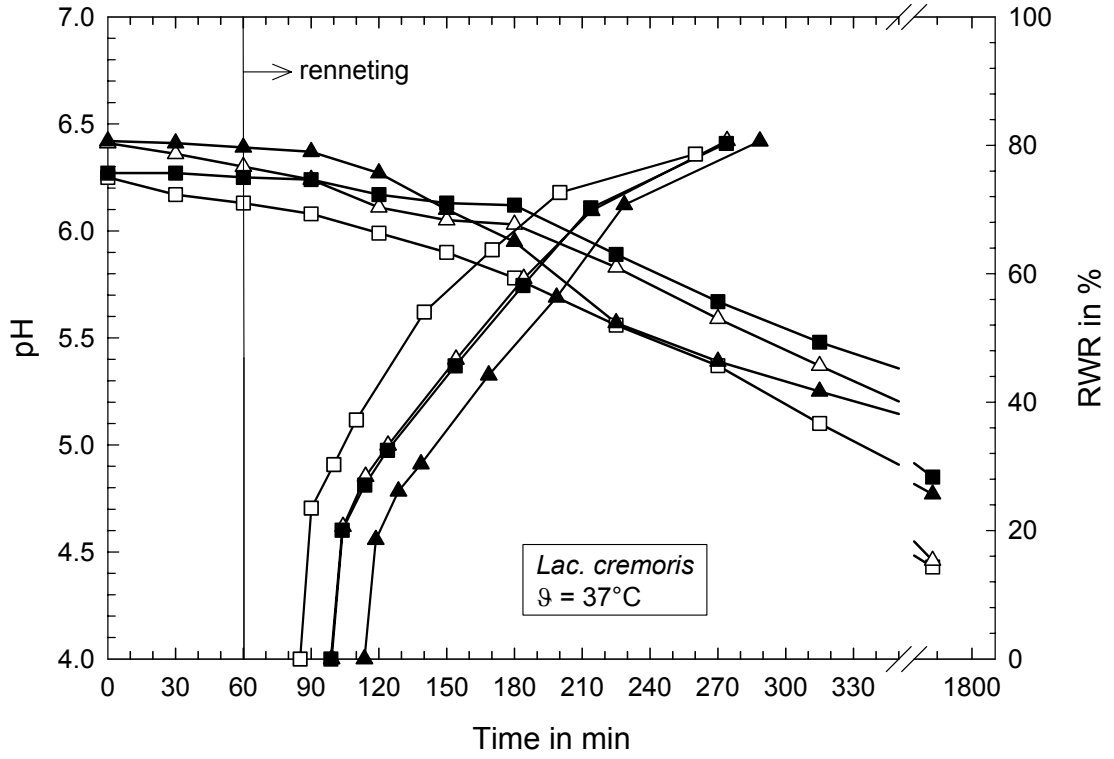

Figure 3.2: Progression of fermentation and syneresis after addition of different amounts of fermented medium (FM_{EPS}) of *Lac. cremoris* at 37 °C. (Δ: 5 % FM_{EPS}; ▲: 5 % FM_{EPS}th; □: 10 % FM_{EPS}; ■: 10 % FM_{EPS}th; th: thermisation).

Finally, plotting syneresis values against the corresponding pH values from fermentation experiments enables to evaluate which trial is generally applicable for soft cheese manufacture. This is demonstrated exemplarily for trials with *Lac. cremoris* in Figure 3.3. The target values for syneresis and pH define an area in which the plotted curves should range. In the case of *Lac. cremoris*, only 10 % FM_{EPS}th fits this objective. The other trials were not applicable for cheesemaking because acidification was too fast and syneresis was too slow. Coagulation time for trials with 5 % FM_{EPS} and 5 % FM_{EPS}th was delayed due to a higher pH value at renneting. The longer the coagulation time the lower the pH at cutting time due to active starter culture acidifying the curd. Although syneresis is accelerated at a lower pH (Lodaite *et al.* 2000; Piyasena and Chambers 2003), the strong drop in pH was not compensated. Thus, syneresis and decrease in pH did not match in these cases. For *Lb. sakei*, trials with 5 and 10 % FM_{EPS}th were applicable for soft cheese manufacture (results not shown).

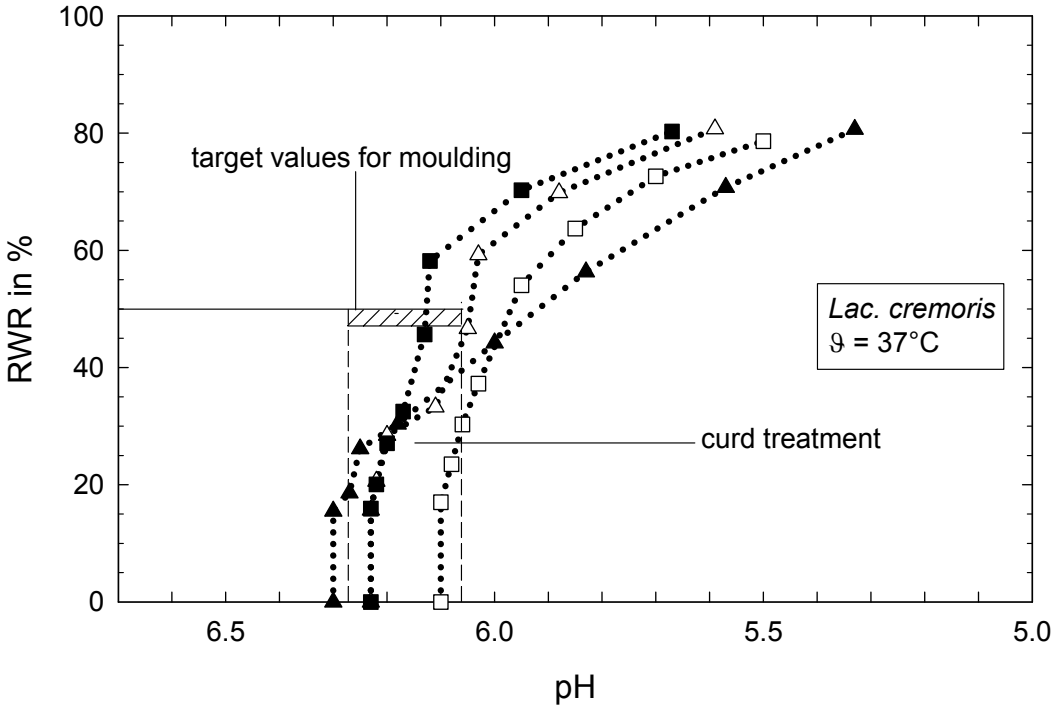

Figure 3.3: Syneresis plotted against pH for milk supplemented with different amounts of fermented medium (FM_{EPS}) of *Lac. cremoris* at 37 °C. (△: 5 % FM_{EPS}; ▲: 5 % FM_{EPS}th; □: 10 % FM_{EPS}; ■: 10 % FM_{EPS}th; th: thermisation).

Based on the determined schedules, soft cheeses supplemented with FM_{EPS} were produced (Table 3.2). Soft cheese produced according to the improved process schedule appeared comparable to standard without sensory defects. Ripening was

not impaired. The dry matter of raw cheese with 10 % FM_{EPS}th of *Lac. cremoris* and *Lb. sakei* was 43.01 and 44.14 %, compared to 46.54 % of the standard. The fat and protein content of raw cheese with 10 % FM_{EPS}th of *Lac. cremoris* (*Lb. sakei*) was 24.22 % (24.81 %) and 20.93 % (21.51 %), compared to 26.94 % and 22.62 % of the standard. The addition of FM_{EPS}th increased the moisture content of soft cheeses, but process conditions had to be carefully adapted. In agreement with results obtained for low-fat Mozzarella cheese and Cheddar cheese (Perry *et al.* 1997; Low *et al.* 1998; Perry *et al.* 1998; Bhaskaracharya and Shah 2001; Dabour *et al.* 2005), moisture content of soft cheese was increased by addition of EPS-producing cultures.

Table 3.2: Process schedules for soft cheese manufacture at 37 °C depending on concentration and treatment of EPS-containing fermented medium (FM_{EPS}).

Strain	FM_{EPS} (%)	th	$t_{preripening}$ (min)	$pH_{renneting}$	$t_{gelling\ point}$ (min)	$t_{coagulation}$ (min)	$t_{curd\ treatment}$ (min)	$pH_{moulding}$	$pH_{chilling}$
Standard	*	-	60	6.51	18.0	60	55	6.19	5.15
Lac. cremoris	5	-	60	6.30	8.3	33	68	6.05	5.18
	10	-	60	6.08	4.8	19	51	5.99	5.10
	5	+	60	6.36	11.9	48	62	6.02	5.09
	10	+	60	6.22	8.2	33	72	6.14	5.16
Lb. sakei	10**	-	40	6.13	5.0	25	45	6.08	5.37
	5	+	56	6.38	11	44	60	6.03	5.15
	10**	+	55	6.20	6.6	27	62	6.04	5.15

*: addition of 10 % skim milk; **: addition of FM_{EPS} after preripening; th: thermisation

Starting from the problem that due to the addition of FM_{EPS} soft cheese manufacture was not feasible under standard conditions, the processing was technologically adapted. By means of plotting syneresis values against pH values, it was possible to read out schedules for each successful processing of soft cheese with a supplement of EPS-producing cultures.

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4 COMPARISON OF MODELS FOR THE KINETICS OF SYNERESIS OF CURD GRAINS MADE FROM GOAT'S MILK

Abstract

The syneresis of curd grains made from Dahlem Cashmere (DC) *goat's milk with edge lengths of 4 and 11 mm was followed with the Dynamic Model System (D.M.S.) at temperatures from 25 to 60 °C. The higher the temperature and the smaller the grains, the more whey was released. Three mathematical models were compared for their suitability describing temperature-induced syneresis and providing kinetic parameters. The kinetic parameters obtained by a linearised model analogue to Michaelis-Menten gave best curve fittings to the experimental data with high coefficient of correlation. In contrast to formal kinetics of non-first order only two constants had to be calculated instead of three. The activation energy (E_A) of temperature-induced syneresis for curd grains of 4 and 11 mm edge length was about 50 kJ mol^{-1} for the linearised model. In contrast, applying non-first order kinetics, E_A varied with curd grain size.*

Keywords: *Syneresis; Dahlem Cashmere; Formal Kinetics*

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4.1 Introduction

Syneresis is a phenomenon that occurs spontaneously after cutting rennet curd into grains. The endogenous syneresis pressure of a rennet gel turns out to be exceptionally small, i.e. about 1 Pa (Walstra *et al.* 1999). Thus, it is normal practice in cheesemaking to enhance syneresis by exerting mechanical treatments like cutting, stirring and others. Vliet and Walstra (1994) reported that about 90 % of the water present in milk gels is mechanically immobilized between the casein strands forming the network, and most of the other water is associated with the casein micelles. Therefore, the ease of whey release after cutting the curd depends principally on the structure of the casein grains and hardly on the hydration of the proteins.

Modification in the composition of the cheese milk results more or less in a changed casein network and thus influences the ability of expelling whey out of the three-dimensional matrix. The rate and quantity of syneresis are affected by biochemical effects and the applied technology. A multitude of investigations showed that the extent of syneresis depends on factors like the composition of the milk, calcium equilibria, the casein concentration, the fermentation rate, temperature, the gel firmness at cutting time and the surface area of the curd grain (Patel *et al.* 1972; Lelievre 1977; Walstra *et al.* 1985; Casiraghi *et al.* 1987; Casiraghi *et al.* 1989; Renault *et al.* 1997; Daviau *et al.* 2000; Grundelius *et al.* 2000; Lodaite *et al.* 2000; Huber *et al.* 2001; Piyasena and Chambers 2003).

However, automatisation of cheesemaking with high-standardized quality and low deviation in weight and composition demands a better description of syneresis as a function of milk composition and technological treatment, particularly when new process steps, i.e. concentration of the milk, is introduced. Thus, the description of syneresis of rennet curd grains as a time-dependent process by means of a mathematical model is essential to estimate and predict whey removal during processing.

Most of the authors describe syneresis as a first order reaction (Kirchmeier 1972; Marshall 1982; Kaytanli *et al.* 1994; Bueeler *et al.* 1997; Calvo and Balcones 2000; Castillo *et al.* 2000). El-Shobery and Shalaby (1992) reported a second order reaction for syneresis of buffalo rennet curd at different temperatures, where the inverse of the released whey was plotted against time t. Huber *et al.* (2001) modelled

the data of thermally-induced syneresis by formal kinetics. In contrast to most other authors they calculated a reaction order of 5. They expected a reaction of third order since the contraction is a three-dimensional process. Due to the fact that the three-dimensional network is flexible during syneresis and new linkages are continuously built up or fractured, the amount of 5 could be attributed to this.

In summary, some different models exist to describe syneresis. According to Ramet and Scher (2000) a general model for the prediction of syneresis from data produced from various parameters of coagulation and syneresis does not currently exist. Consequently, the objective of this study was to present a mathematical model for the prediction of syneresis and to compare it to the calculation method of Huber *et al.* (2001) and to kinetics analogue to Michaelis-Menten.

Conflicting results are found in the literature because of different methods used following the contraction and whey release of rennet curd grains. The results are thus not comparable among each other. Furthermore, syneresis was not followed under dynamic conditions. Most syneresis experiments reported in the literature were carried out with bovine milk. Due to the lack of syneresis data for caprine rennet curd grains, milk of Dahlem Cashmere (DC) goats was taken for the experiments. Dimassi *et al.* (2005) demonstrated the potential of milk of DC goats for cheese production. In order to compare cheesemaking properties of DC goat's milk with bovine milk, the experiments were carried out to generate valuable data for describing kinetics of syneresis of curd grains.

4.2 Materials and Methods

4.2.1 Milk Samples and Sample Preparation

Raw bulk milk of DC goats was provided by the research station Oberer Lindenhof (University of Hohenheim, Germany). Experiments were carried out over a period of three weeks. The milk was batch-wise skimmed at 50 °C using a disc separator (Type Elecrem, HÄKA Buttermaschinen GmbH, Stutensee, Germany), pasteurized at 63 °C for 30 min, cooled down to 6 °C, and stored in a cooling chamber at 6 °C. The average composition of the milk was 3.52 ± 0.06 % for protein, 2.91 ± 0.05 % for casein and 9.35 ± 0.13 % for dry matter content. After addition of calcium chloride

(1.02382.1000, Merck, Darmstadt, Germany) in a concentration of 0.02 % (w/w), the milk was stirred and gently warmed up to 30 °C before syneresis experiments. pH_{20} = 6.50 was adjusted with 9 % lactic acid (1.00366, Merck, Darmstadt, Germany) at 30 °C (pH_{20}: calibration of the pH-electrode (pH 522, SCHOTT, Mainz, Germany) was performed at 20 °C and the measurement was done at the appropriate temperature without automatic temperature correction). The adjusted milk was portioned in parts of 100 g and tempered at 30 °C in a water bath (WB14, Memmert GmbH & Co KG, Schwabach, Germany). After addition of 0.02 % (v/w) chymosin (strength 1:15000, Chymosin ≥ 99 %, Chr. Hansen, Hoersholm, Denmark) the sample was stirred for 1 min and kept till cutting at 30 °C.

4.2.2 Syneresis Test

Syneresis experiments were carried out with the Dynamic Model System (D.M.S.), already used by Huber *et al.* (2001). In principle, at the end of the coagulation time of 60 min the rennet gel was cut in grains with defined edge lengths of 4 mm, respectively 11 mm, representing curd grain size of hard and semi-hard cheeses. Centrifuge cups (DURHAN tube with screw thread, DIN 12216, VWR, Darmstadt, Germany) were filled with 50 mL sweet whey (5.2 % dry matter, pH 6.3), reconstituted from spray-dried sweet whey powder (Schwarzwaldmilch, Offenburg, Germany) with distilled water. Six curd grains with an edge length of 4 mm and four grains with an edge length of 11 mm, respectively, were transferred to the cups filled with sweet whey, tempered in incubators (WTR-1, Infors AG, Bottmingen-Basel, Switzerland and Incubator Shaker Model G25, New Brunswick Scientific Co. Inc, Edison, U.S.A.). The frequency of the incubators was set to 200 rpm. The thermal treatment ranged from 25 to 60 °C. The vibration times were in general 5, 10, 20, 30, 60, 90, 120 and 240 minutes. The relative whey removal, RWR, indicating the time-dependent syneresis, was calculated from the initial weight of the grains, m_0, and the weight of the grains after treatment, m_t.

$$RWR = \left(1 - \frac{m_t}{m_0}\right) \cdot 100 \qquad (4.1)$$

RWR: relative whey removal in %; m_0: initial mass of curd grains in g at time t = 0; m_t: mass of curd grains in g after time t

The initial weight, m_0, of the curd grains at time t = 0 was determined by weighing the centrifuge cups before and after adding the grains. After time t, one of the cups was taken out of the incubator, the grains and the whey were poured on a grit (pore size of approximately 0.5 mm) to separate the whey from the shrunken grains. With a paper towel, the adsorbed whey in the grit's pores was removed and the grains were immediately weighed, m_t. This step was introduced to remove also the whey layer that is bound to the surface of the grain. Sucking out whey due to capillary effects has to be avoided. This was taken care of by shortly striving the paper towel only once crosswise over the grit's rear side. Four replicates were performed of each sample.

4.2.3 Modelling of Syneresis

According to Huber *et al.* (2001) the time-dependent process of syneresis was described by formal kinetics. The mass loss, dm, of the initial mass, m, of the rennet curd grains that occurs in the time element, dt, by the release of whey is expressed in equation 4.2.

$$\frac{dm}{dt} = -k \cdot m^n \tag{4.2}$$

n: order of reaction; k: temperature dependent rate constant in s^{-1}

Integration of equation 4.2 results for an order of reaction ≠ 1 in equation 4.3

$$\frac{m_t}{m_0} = \left[1 + (n-1) \cdot k \cdot t\right]^{\frac{1}{1-n}} \tag{4.3}$$

and for n = 1 in equation 4.4

$$\frac{m_t}{m_0} = \exp(-k \cdot t) \tag{4.4}$$

in which m_0 represents the initial mass of the curd grains and m_t the grains mass at time t. The influence of the temperature, T, on the rate constant of the reaction is estimated by the well-known Arrhenius equation. The measured data did not well fit by using a reaction order of n = 1. Thus, the data were evaluated only by non-first

order shown in equation 4.3. After inserting equation 4.3 in the Arrhenius equation, equation 4.5 is obtained.

$$\left(\frac{m_t}{m_0}\right) = \left[1 + (n-1)\cdot k_{T_{ref}} \cdot \exp\left(-\frac{E_A}{R}\cdot\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right)\cdot t\right]^{\frac{1}{1-n}} \qquad \text{for } n \neq 1 \qquad (4.5)$$

$k_{T_{ref}}$: rate constant at reference temperature in s^{-1}; E_A: activation energy in J mol^{-1}; universal gas constant R = 8.314 J $(mol\ K)^{-1}$; t: time in s; T: absolute temperature in K; reference temperature T_{ref} = 308 K

Insertion of equation 4.1 in equation 4.5 generates equation 4.6, which was the first model applied for the description of syneresis. In the following, equation 4.6 is referred to as model 1.

$$RWR = \left(1 - \left[1 + (n-1)\cdot k_{T_{ref}} \cdot \exp\left(-\frac{E_A}{R}\cdot\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right)\cdot t\right]^{\frac{1}{1-n}}\right)\cdot 100 \quad \text{for } n \neq 1 \quad (4.6)$$

RWR: relative whey removal in %; $k_{T_{ref}}$: rate constant at reference temperature in s^{-1}; E_A: activation energy in J mol^{-1}; universal gas constant R = 8.314 J $(mol\ K)^{-1}$; t: time in s; T: absolute temperature in K; reference temperature T_{ref} = 308 K

The curve progression of temperature-induced syneresis is comparable to kinetics of enzyme reactions. Hence, the second model applied for describing syneresis is analogue to Michaelis-Menten kinetics. Equation 4.7 shows the equation where the Michaelis constant, K_M, and the maximum rate constant, v_{max}, are replaced by the constants $t_{1/2}$ and RWR_{max}, respectively. In the following, equation 4.7 is referred to as model 2a. $t_{1/2}$ is the time after that half of the maximum whey amount of a curd grain is released. RWR_{max} is the maximum whey amount that is released after infinite time.

$$RWR = \frac{RWR_{max}\cdot t}{t_{1/2} + t} \qquad (4.7)$$

The kinetic parameters were calculated by non-linear regression.

The third model for description of syneresis is also based on equation 4.7, but the kinetic parameters are estimated by linearisation of the experimental data (eq. 4.8). In the following, equation 4.8 is referred to as model 2b.

$$\frac{t}{RWR} = \frac{1}{RWR_{max}} \cdot t + \frac{t_{1/2}}{RWR_{max}} \tag{4.8}$$

The kinetic parameters were recorded through regression with the software Sigma Plot 8.0 (SPSS Inc., Chicago, USA). Non-linear coefficients of correlation, r^2_{nl}, were obtained for model 1 (eq. 4.6) and model 2a (eq. 4.7) whereas linear coefficients, r^2, were obtained for model 2b (eq. 4.8).

4.3 Results

Figure 4.1 shows the relative whey removal of DC curd grains with an edge length of 11 mm as a function of vibration time for various temperatures.

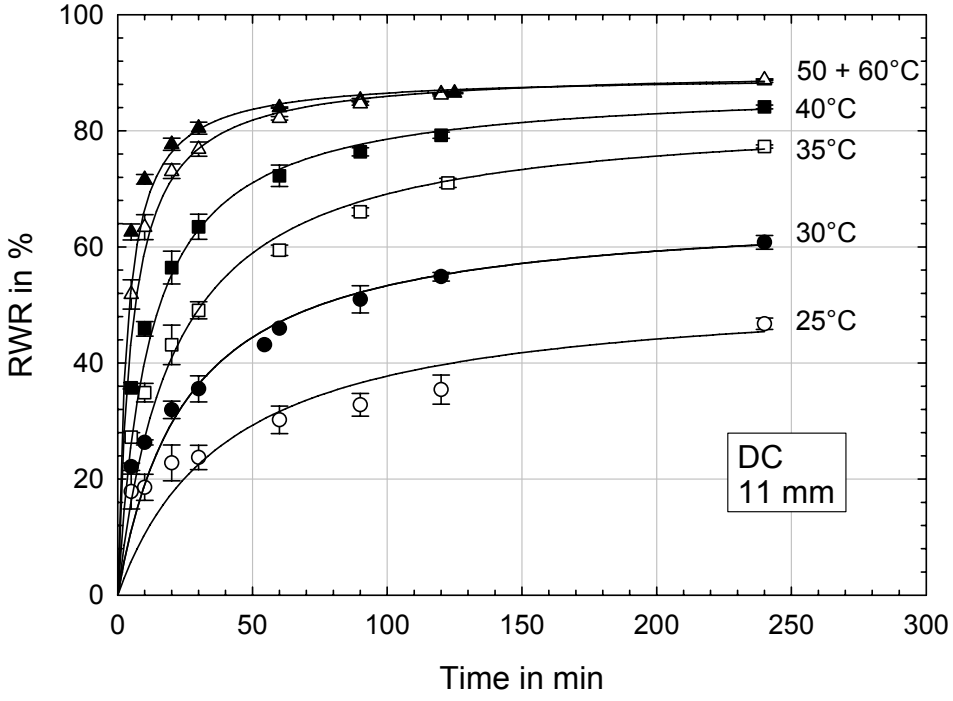

Figure 4.1: Relative whey removal (RWR) of DC curd grains with an edge length of 11 mm as a function of time for 25 °C (○), 30 °C (●), 35 °C (□), 40 °C (■), 50 °C (Δ) and 60 °C (▲). Plotted are the mean values of four measurements with standard deviation and the calculated lines according to parameters from model 2b (Table 4.3).

The progression of syneresis for curd grains of 4 mm edge length for various temperatures is comparable (data not shown). With extended time and whey temperature starting from 25 °C, syneresis increased. Above 50 °C and within a vibration time of approximately 90 min, syneresis was still accelerated but decreased to the level of the 50 °C syneresis curve. It is obvious that within the first 30 min most of the serum of the curd grains was already expelled.

4.3.1 Formal Kinetic Parameters for Temperature-induced Syneresis

Table 4.1 represents the formal kinetic parameters calculated by means of model 1 (eq. 4.6) for rennet curd grains with an edge length of 4 and 11 mm. The parameters differed with curd grain size and were in principal higher for the smaller grains. The amount of E_A, being 160.7 and 138.9 kJ mol^{-1}, respectively indicates the dependency of syneresis on temperature. The rate constant, $k_{T_{ref}}$, was lower for an edge length of 11 mm compared to 4 mm. The amount of n, being 4.6 and 5.87, respectively, was higher then cited in the literature.

Table 4.1: Formal kinetic parameters calculated according to model 1 (eq. 4.6) with standard error (s.e.) for temperature-induced syneresis within 25 to 60 °C of DC rennet curd grains with an edge length of 4 and 11 mm.

Grain size in mm	$E_A \pm$ s.e. in kJ mol^{-1}	$k_{T_{ref}} \pm$ s.e. in s^{-1}	n $\pm$ s.e. [-]	r^2_{nl}	Number of samples
4	160.7 $\pm$ 7.8	(3.15 $\pm$ 0.52) $\cdot$ 10^{-4}	5.87 $\pm$ 0.20	0.965	155
11	138.9 $\pm$ 4.9	(2.67 $\pm$ 0.23) $\cdot$ 10^{-5}	4.60 $\pm$ 0.16	0.955	199

E_A: activation energy; $k_{T_{ref}}$: rate constant at reference temperature (308 K); n: order of reaction; r^2_{nl}: non-linear coefficient of correlation

Nevertheless, the model allows the description of syneresis for both edge lengths, covering a wide temperature range, with good correlation, as the non-linear coefficient of correlation with amounts above 0.95 demonstrates. The quality of the modelling is exemplarily illustrated in Figure 4.2 for curd grains with an edge length of 11 mm. The graph for grains with 4 mm edge length is comparable (data not shown). Experimental and calculated data should be equally distributed along the bisector. At high RWR, the data are well described by the model, but below 50 % RWR the data are mostly above and below 25 % RWR mostly below the bisector. This indicates that the model does not describe RWR below 50 % very well, although the coefficient of correlation is high.

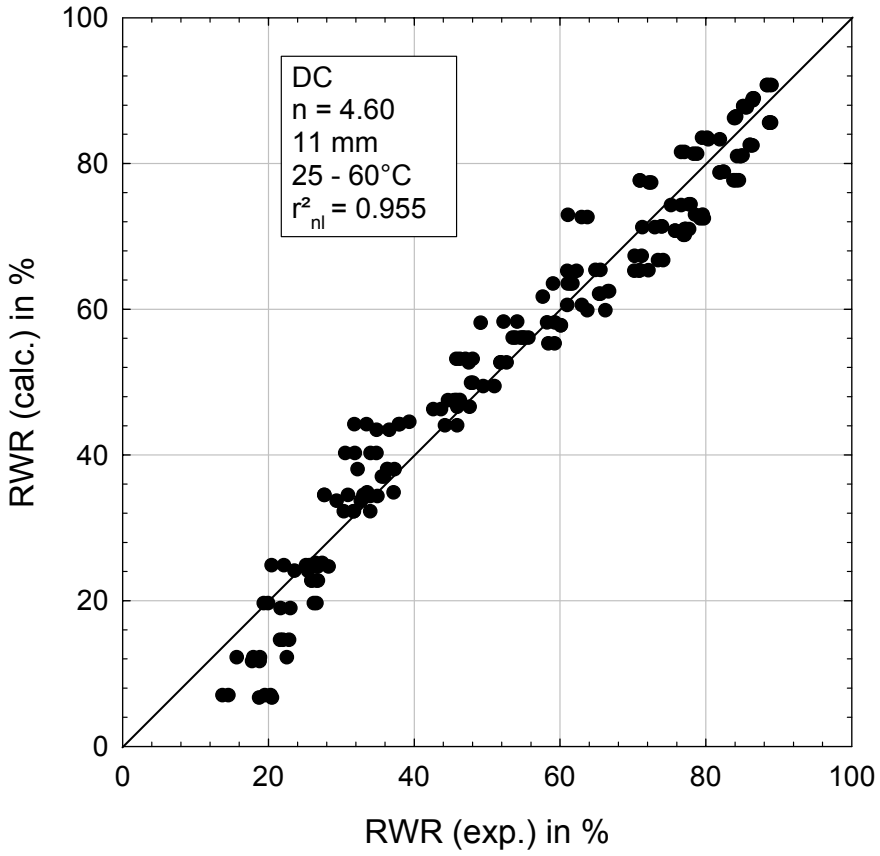

Figure 4.2: Correlation between experimental data and calculated data (model 1, eq. 4.6) for the syneresis of DC curd grains with an edge length of 11 mm under dynamic conditions for temperatures from 25 to 60 °C.

4.3.2 Kinetic Parameters for Temperature-induced Syneresis obtained by means of Model 2a and 2b

Since model 2b is based on model 2a, the results of both models are presented in this section. Table 4.2 and Table 4.3 depict the kinetic parameters for grains with an edge length of 4 and 11 mm, respectively, that were obtained using model 2a (eq. 4.7) and model 2b (eq. 4.8), respectively. Added are the amounts of RWR after 240 min. Contrary to model 1 (eq. 4.6), the parameters were calculated for each temperature. RWR_{max} is the whey release after infinite time and $t_{1/2}$ is the time after that half of RWR_{max} is released. RWR_{max} appears as an important value because it describes the endpoint of syneresis depending on changes in process technology or in the composition of the milk.

Table 4.2: Kinetic parameters with standard error (s.e.) for syneresis of DC rennet curd grains with an edge length of 4 mm, depending on the temperature.

Temp. in °C	Model 2a (eq. 4.7)			Model 2b (eq. 4.8)			RWR_{240} in %	Number of samples
	$RWR_{max} \pm$ s.e. in %	$t_{1/2} \pm$ s.e. in s	r^2_{nl}	$RWR_{max} \pm$ s.e. in %	$t_{1/2} \pm$ s.e. in s	r^2		
30	66.6 ± 2.9	207 ± 63	0.95	75.4 ± 1.2	625 ± 98	> 0.99	74.0	33
35	75.7 ± 2.1	207 ± 40	0.98	81.9 ± 0.9	459 ± 65	> 0.99	80.5	33
40	81.2 ± 1.5	132 ± 22	0.99	84.4 ± 0.4	243 ± 25	> 0.99	83.2	32
50	87.12 ± 1.01	67 ± 10	> 0.99	89.6 ± 0.3	129 ± 16	> 0.99	89.0	28
60	88.12 ± 0.91	35.9 ± 8.2	> 0.99	90.6 ± 0.2	96 ± 13	> 0.99	90.2	29

RWR_{max}: relative whey removal after infinite time; $t_{1/2}$: time after that half of RWR_{max} is expelled; r^2_{nl}: non-linear coefficient of correlation; RWR_{240}: relative whey removal after 240 min

Table 4.3: Kinetic parameters with standard error (s.e.) for syneresis of DC rennet curd grains with an edge length of 11 mm, depending on the temperature.

Temp. in °C	Model 2a (eq. 4.7)			Model 2b (eq. 4.8)			RWR_{240} in %	Number of samples
	$RWR_{max} \pm$ s.e. in %	$t_{1/2} \pm$ s.e. in s	r^2_{nl}	$RWR_{max} \pm$ s.e. in %	$t_{1/2} \pm$ s.e. in s	r^2		
25	39.8 ± 1.4	756 ± 130	0.72	46.5 ± 1.4	1382 ± 180	0.95	46.8	44
30	61.0 ± 1.7	963 ± 100	0.92	64.8 ± 1.0	1204 ± 110	> 0.99	60.8	31
35	77.1 ± 1.6	805 ± 68	0.95	81.6 ± 0.9	1024 ± 76	> 0.99	77.3	31
40	84.4 ± 0.9	529 ± 19	0.97	87.3 ± 0.5	654 ± 35	> 0.99	84.1	30
50	88.3 ± 0.5	230 ± 8	0.98	90.3 ± 0.2	297 ± 14	> 0.99	88.8	32
60	87.5 ± 0.4	129 ± 5	0.97	89.5 ± 0.2	195 ± 15	> 0.99	88.7	31

RWR_{max}: relative whey removal after infinite time; $t_{1/2}$: time after that half of RWR_{max} is expelled; r^2_{nl}: non-linear coefficient of correlation; RWR_{240}: relative whey removal after 240 min

For both grain sizes, RWR_{max} increased and $t_{1/2}$ decreased with increased temperature and confirmed the matter of fact, that applying high temperatures during cheese processing enhance syneresis. Grains with an edge length of 11 mm reached the maximum amount of RWR_{max} at 50 °C. A further rise in temperature accelerated the whey release at the beginning of syneresis, indicated by a lower amount of $t_{1/2}$ (195 s at 60 °C compared to 297 s at 50 °C), but hardly influenced RWR_{max}.

The kinetic parameters obtained by model 2b (Table 4.2 and 4.3) are higher than those obtained by model 2a. The amount of RWR after 240 min was similar to the amount of RWR_{max} received by model 2b. Regarding the coefficients of correlation, model 2b allowed especially for 25 and 30 °C a better fit to the measured data. The plotted curves in Figure 4.1 were calculated using the kinetic parameters obtained by means of model 2b (Table 4.3) and inserted in equation 4.7.

4.3.3 Calculation of E_A from the Kinetic Parameter $t_{1/2}$

Since the kinetic parameter $t_{1/2}$ represents the time for half of the maximum whey release, its reciprocal amount may be equated with the rate constants used for the Arrhenius plot. The Arrhenius plot of the logarithmic reciprocal constant $t_{1/2}$, obtained by model 2b, delivered the values E_A and $k_{T_{ref}}$ that are shown for both grain sizes in Table 4.4.

Table 4.4: Activation energy (E_A) and rate constant at reference temperature for temperature-induced syneresis within 25 to 60 °C of DC rennet curd grains with an edge length of 4 and 11 mm. The parameters were calculated using the data of $t_{1/2}$ obtained by model 2b.

Grain size in mm	$E_A \pm$ s.e. in kJ mol^{-1}	$k_{T_{ref}} \pm$ s.e. in s^{-1}	r^2
4	54.7 ± 6.4	$(2.18 \pm 0.36) \cdot 10^{-3}$	0.96
11	50.1 ± 4.2	$(9.78 \pm 0.77) \cdot 10^{-4}$	0.97

$k_{T_{ref}}$: rate constant at reference temperature (308 K), s.e.: standard error

The amount of E_A of both grain sizes was in the same range and did not differ significantly. The rate constant increased with decreasing grain size, which is in qualitative agreement with the parameter in Table 4.1.

4.4 Discussion

4.4.1 Formal Kinetic Parameters for Temperature-induced Syneresis

As already mentioned, syneresis was often described as a first order reaction (Kirchmeier 1972; Kaytanli *et al.* 1994; Bueeler *et al.* 1997; Calvo *et al.* 2000) what is in contrary to results by Huber *et al.* (2001) and our results. For bovine rennet curd grains of 11 mm size Huber *et al.* (2001) determined within 25 and 50 °C an E_A = 108 kJ mol^{-1} and a $k_{T_{ref}} = 2.66 \cdot 10^{-5}$ s^{-1}, applying model 1. The kinetic parameters of DC rennet curd grains with an edge length of 11 mm presented in Table 4.1 are comparable.

In contrast, kinetic parameters of both analyzed grain sizes were not comparable. The rate constant for the smaller grains was about one order of magnitude higher than for the grains of 11 mm curd size. These observations confirmed reports of Walstra *et al.* (1985) and Renault *et al.* (1997) that syneresis was proportional to the

area of curd particles. The smaller the curd grain, the higher the surface to volume ratio, so that due to cutting the permeability of the three-dimensional system increases. More pores are opened in which whey is mechanically entrapped. According to Darcy's law, the superficial velocity of a liquid through a porous material is not only dependent on the permeability of the system but also on the distance that the liquid flows through. In other words, cutting the curd in smaller grains reduces the distance for effluent whey and thus syneresis is accelerated. Despite the changed curd size, temperature-induced syneresis is unmodified and so the reaction should be characterized by the same order of reaction. According to the results in Table 4.1, this was not the case. The amount of n being 5.87 and 4.6, respectively, are difficult to explain and represent only formal parameters not reflecting any basic mechanism.

Regarding Figure 4.2, it seems that the data points are coincidentally arranged around the bisector. Below 25 %, the measured amounts were higher than those obtained by calculation. This indicates that in the early stage of syneresis, especially for low temperatures between 25 and 30 °C, the prediction of whey release by model 1 was not sufficient. Therefore, r^2_{nl} does not reflect the fitting to the individual data set of one temperature.

The advantage of model 1, providing only three parameters that can be applied over a wide range of temperature, is reduced by the fact that the model did not fit the experimental data for an individual temperature well.

4.4.2 Kinetic Parameters for Temperature-induced Syneresis obtained by means of Model 2a and 2b

Above 50 °C, the whey release of the outer parts of the grains is faster than the whey flow from the inner to the outer parts. In consequence, due to skin formation (Kammerlehner 2003), RWR_{max} in Table 4.3 was smaller for 60 °C than for 50 °C, reflecting the hindered syneresis due to skin formation. $t_{1/2}$ represents the rate of syneresis and should become smaller with increasing temperature. Except for 25 °C the data in Table 4.2 and 4.3 confirm this expectation. Due to the low amount of RWR_{max} for 25 °C, obtained by model 2a, half of RWR_{max} was sooner released than for 30 °C resulting in a lower amount of $t_{1/2}$. The kinetic data obtained by model 2b did not show this expectation and their amounts were generally slightly higher.

Above 35 °C both models fitted the experimental data well, whereas r^2 for model 2b was always higher. Only for a temperature of 25 °C the plotted curve in Figure 4.1 differed from syneresis data. Kirchmeier (1972) calculated a limiting temperature of 16 °C below that no syneresis should occur. According to this, a temperature of 25 °C may be high enough to force syneresis with ongoing time. However, factors like formation of a liquid layer around the curd grain or the susceptibility of the grains to fracture may influence the determination of syneresis, so that the calculated and plotted curve differed from experimental data points.

Regarding the values in Table 4.2, we find a discrepancy between RWR measured after 240 min, RWR_{240}, and RWR_{max} for model 2a. At 35 °C, 80.5 % of whey was released after 240 min, whereas a RWR_{max} was calculated of about 75.7 %. It has to be mentioned that the error of measuring syneresis for extended time in the D.M.S. was always low. Therefore, the quality of the mathematical model is to be proved for its suitability fitting also to syneresis data measured after long treatment. As a result, the kinetic data calculated according to model 2b allow a curve fitting that satisfies the main criteria: the modelling of whey release over the total time of measurement is of excellent quality.

4.4.3 Calculation of E_A from the Kinetic Parameter $t_{1/2}$

The amounts of E_A shown in Table 4.1 where E_A was calculated by means of model 1 were with 138.9 and 160.7 kJ mol^{-1} much higher than those shown in Table 4.4, but in agreement with the results of Huber *et al.* (2001). They explained an E_A being approximately 100 kJ mol^{-1} by the theory, that a chemical reaction like the formation of non-covalent bonds was responsible for the shrinkage of the curd grains.

According to Westphal *et al.* (1996), E_A for chemical reactions is between 50 and 105 kJ mol^{-1}. E_A in Table 4.4, determined with model 2b, were within the described range and did not significantly differ for curd grains with edge lengths of 4 and 11 mm. Although being in the defined E_A-interval for chemical reactions according to Westphal *et al.* (1996), the value was still in the lower threshold. Despite the statement of Walstra *et al.* (1999) that syneresis is not a diffusion process, an E_A of about 50 kJ mol^{-1} is revealing of an overlap of two reactions: formation of new bonds and diffusion. Kessler (2002) and Westphal *et al.* (1996) defined for diffusion an E_A of about 10 - 20 kJ mol^{-1}. Furthermore, this amount corresponds to an E_A for

temperature-induced decrease of the viscosity of water. With decreasing serum viscosity, the laminar flow in pores is increased.

Altogether, the similar amount of E_A obtained by means of model 2b (eq. 4.8) indicates that the model describes syneresis well, independent from curd grain size.

4.5 Conclusion

The D.M.S. is a suitable standardized method to follow syneresis of curd grains with sizes of 4 and 11 mm. Temperature-induced syneresis within 25 and 60 °C was best fitted with the kinetic data obtained by model 2b. By means of only two parameters, excellent curve fitting was possible ($r^2 \geq 0.99$). With RWR_{max}, the model provides a kinetic parameter that gives information about the endpoint of syneresis. Therefore, it is recommended that the calculation of kinetic data should be performed according to model 2b. Further studies to evaluate syneresis and thus cheesemaking properties of DC goat's milk comparing to milk of other animal species are already in process.

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5 STRATEGY TO EVALUATE CHEESEMAKING PROPERTIES OF MILK FROM DIFFERENT GOAT BREEDS

Abstract

Syneresis experiments were conducted on skim milk from two different goat breeds (Dahlem Cashmere, DC; German White, GW) in order to evaluate its cheesemaking potential. The conditions were those applied in semi-hard cheese production and bovine milk was used as a control. The influences of temperature (25 to 60 °C) and of curd grain size (11 and 22 mm) on syneresis were investigated. The syneresis data were described by a kinetic model as a function of the curd incubation time (up to 240 min). Chemical analyses and renneting properties of the milk were also studied. RWR_{max} *(maximum whey removal after infinite time) for curd grain sizes of 11 and 22 mm showed a significant difference between DC and GW milk, and between bovine and GW milk. No significant differences were found between bovine and DC milk. Firmness of the gels after 60 min of coagulation, dry matter, protein and casein contents of DC milk were significantly higher (*$P < 0.05$*) than GW milk and similar to bovine milk. The strategy applied in this study can be used to evaluate and compare cheesemaking properties of different milks. In addition, it was demonstrated that DC goat's milk is suitable for cheesemaking, even under conditions applied in semi-hard cheese manufacture using bovine milk.*

Keywords: *Goat Milk; Rennet Coagulation Time; Curd Firmness; Syneresis*

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5.1 Introduction

About 1.5 mio tonnes of goat's milk were produced in the European Union in 2001 (Medina and Nuñez 2004). Notably in France, the largest producer of goat's milk in the world, more than 90 % of goat milk is processed and sold as cheese (Dubeuf *et al.* 2004). Due to the composition of goat's milk and the limited lactation period, cheese is often made from mixtures of cow's, ewe's and goat's milk (McSweeney *et al.* 2004). However, the production of mixed milk cheeses in France amounts only to 5 % of the total goat's cheese production and their share is decreasing year after year (Le Pape and Priou 2005).

Regarding the diversity of cheese types using goat's milk, less cheese types exist compared to cheeses made from bovine milk and most cheeses fall into the group of fresh or white unripened cheeses and soft cheeses. This is mostly related to the poor mechanical properties of goat's milk curd, which is generally too soft to resist the applied mechanical forces during curd treatment in semi-hard and hard cheese manufacture (Medina and Nuñez 2004). Besides a lower casein content in goat's milk compared to bovine milk, the main factor responsible for its technological limitations is the composition of the goat's milk casein. In particular, caprine casein contains a lower proportion of α_{s1}-casein and a higher proportion of β-casein than bovine milk. Cheesemaking from goat's milk with a low α_{s1}-casein content resulted in a less firm curd and a lower cheese yield compared to milk with high α_{s1}-casein content (Ambrosoli *et al.* 1988). Dimassi (2005) showed that even when milk from high and low α_{s1}-casein breeds were standardized to the same level of solid non fat, the milk from higher α_{s1}-casein resulted in better cheese conversion values.

Any change in milk composition strongly influences coagulation properties and thus the further process steps in cheesemaking, in particular syneresis. Especially, in semi-hard and hard cheese manufacture, syneresis is enhanced by curd scalding and likewise by mechanical treatment like cutting and extensive stirring. Hence, milk used for semi-hard and hard cheese manufacture should generate a curd that resists mechanical forces to avoid cheese dust. An overview of factors which influence syneresis in rate and final value was given by Walstra *et al.* (1985). Storry *et al.* (1983) and Calvo and Balcones (2000) studied the effect of the animal species on

syneresis. They found that the influence of the species on syneresis was a significant factor.

The objective of this work was to investigate a strategy for evaluating the cheesemaking properties of milk from two goat breeds under conditions applied in semi-hard cheese manufacture using bovine milk.

5.2 Materials and Methods

5.2.1 Milk Samples, Sample Preparation and Coagulation

Raw milk of German Holstein cows and milk of Dahlem Cashmere goats (DC) were provided by the Research Station Oberer Lindenhof (University of Hohenheim). Raw milk of German White (GW) goats was purchased from a local farmer. Each experiment was carried out over a period of three weeks. The milk was batch-wise skimmed at 50 °C using a disc separator (Type Elecrem, HÄKA Buttermaschinen GmbH, Germany), pasteurized at 63 °C for 30 min, cooled down to 6 °C, and stored in a cooling chamber at 6 °C until further treatment.

After addition of 0.02 % (w/w) calcium chloride (1.02382.1000, Merck, Germany), the skim milk was gently warmed up to 30 °C before syneresis experiments. A pH_{20} of 6.50 was adjusted with lactic acid (1.00366.2500, Merck, Germany) at 30 °C (pH_{20}: calibration of the pH-electrode was performed at 20 °C and the measurement was done without temperature correction). The milk was portioned in 100 g aliquots and tempered at 30 °C. For coagulation, chymosin (strength 1:15000, chymosin ≥ 99 %, Chr. Hansen, Denmark) was added at a concentration of 0.02 % (v/w). The sample was stirred for 1 min and kept till cutting at 30 °C.

5.2.2 Milk Chemical Composition

The dry matter was determined by means of a standard method according to VDLUFA (2003). Based on the Dumas method DIN 10467, total nitrogen was determined using a Leco FP-528 (Leco Instrumente GmbH, Germany). Total protein was calculated by multiplying the nitrogen content with the milk specific factor of 6.38.

The individual native whey protein fractions were measured by Reverse Phase - High Performance Liquid Chromatography (RP-HPLC) according to the International Dairy Federation (1996). The casein content was calculated according to Kersten (2001).

All analyses were performed in triplicate.

5.2.3 Rheological Measurements

Non-destructive and destructive measurements were carried out to characterize the coagulation properties of the individual milk sample (non-destructive) and the texture of the gels after 60 min of coagulation (destructive). Sample preparation was carried out as described in 5.2.1.

A CS10 controlled-stress rheometer equipped with a double gap device (DG40/50, Bohlin Instruments, Germany) was used for the determination of the rennet coagulation time (RCT). After the addition of rennet, gel formation was monitored at 30 °C by measuring the storage modulus G', the loss modulus G'' and the related phase angle δ at a strain amplitude $\gamma = 0.01$ and frequency (1 Hz). RCT was estimated from the cross-over of the dynamic moduli ($\delta = 45°$). Aggregation rate (AR) was detemined from the slope at maximum increase of G'(t) according to Steffl *et al.* (1999). According to Scott Blair and Burnett (1958), gel-firming kinetics were modelled by equation 5.1.

$$G' = G'_{\infty} \exp\left(-\frac{\tau}{t - RCT}\right) \tag{5.1}$$

G': storage modulus in Pa; G'_{∞}: storage modulus after infinite time; RCT: rennet coagulation time in s; t: time in s; τ: characteristic constant in s

Texture analyses of the gels were performed at 30 °C according to Schreiber and Hinrichs (2000). 60 g of milk was used and the maximum resistance after 60 min of coagulation (F-60-value) was determined using a texture analyzer (Z2.5/TS1S, Zwick GmbH & Co. KG, Germany) equipped with a load cell of 20 N. Each point was determined fivefold.

5.2.4 Syneresis Test

The Dynamic Model System, used by Huber *et al.* (2001), was modified. After 60 min coagulation time the coagulum was cut in cubic grains with defined edge lengths of 11 and 22 mm, being in the range of curd grains typically generated in semi-hard and soft cheese manufacture (Ramet 2000). Centrifugal cups (DURHAN tube with screw thread and cap, DIN 12216, VWR, Germany) were filled with 50 mL sweet whey (5.2 % dry matter, pH_{20} of 6.3), reconstituted from spray-dried sweet whey powder (Schwarzwaldmilch, Germany) with distilled water. Either four grains with an edge length of 11 mm, or one curd grain with an edge length of 22 mm were transferred to the cups filled with sweet whey, tempered in a water incubator (WTR-1, Infors AG, Switzerland). The thermal treatment ranged from 25 to 60 °C. The frequency of the incubator was set to 200 rpm and the treatment times for syneresis were in general 5, 10, 20, 30, 60, 120 and 240 min. The relative whey removal, RWR, indicating the time-dependent syneresis, was calculated from the initial weight of the grains, m_0, and the weight of the grains after treatment, m_t, as follows:

$$RWR = \left(1 - \frac{m_t}{m_0}\right) \cdot 100 \tag{5.2}$$

All syneresis experiments were at least performed in triplicate.

Modelling of syneresis and determination of kinetic parameters were performed according to Thomann *et al.* (2006). Syneresis can be described by equation 5.3.

$$\frac{t}{RWR} = \frac{1}{RWR_{max}} \cdot t + \frac{t_{1/2}}{RWR_{max}} \tag{5.3}$$

RWR_{max}: maximum whey amount that is released after infinite time; t: curd treatment time; $t_{1/2}$: time that half of RWR_{max} is released

The kinetic parameters RWR_{max} and $t_{1/2}$ were calculated by regression using the software Sigma Plot 8.0 (SPSS Inc., USA). Statistical analysis were carried out using ANOVA and the general linear model procedure (SAS 8.0, SAS Institute Inc., Cary, USA) analyzing the influence of breeds and species on RWR_{max} and $t_{1/2}$.

5.3 Results and Discussion

5.3.1 Milk Composition

Ranges in composition of bovine milk and the milk of the two goat breeds are given in Table 5.1.

Table 5.1: Average composition in skimmed bovine, Dahlem Cashmere and German White goat's milk (Mean ± S.D.).

In %	Bovine	Dahlem Cashmere	German White	n
Dry Matter	(9.44 ± 0.12)[a]	(9.36 ± 0.11)[a]	(8.50 ± 0.24)[b]	21
Total Protein	(3.43 ± 0.06)[a]	(3.5 ± 0.1)[a]	(2.79 ± 0.06)[b]	9
Casein	(2.62 ± 0.08)[a]	(2.91 ± 0.05)[b]	(2.12 ± 0.03)[c]	6
Whey Protein	(0.68 ± 0.05)[a]	(0.46 ± 0.03)[b]	(0.572 ± 0.005)[c]	6
α-Lactalbumin	(0.111 ± 0.007)[a]	(0.14 ± 0.01)[b]	(0.156 ± 0.002)[c]	6
β-Lactoglobulin A	(0.25 ± 0.03)	(0.32 ± 0.03)*	(0.417 ± 0.006)*	6
β-Lactoglobulin B	(0.36 ± 0.05)	-	-	6

[a,b] means within a row with different superscripts differ ($P < 0.05$); n: number of samples; S.D.: standard deviation; * only one peak for β-Lactoglobulin

Bovine and DC milk had comparable contents of dry matter and total protein, whereas GW goat's milk had significantly lower values. Dimassi *et al.* (2005) also reported differences in the composition of DC and German Fawn (GF) goat's milk. They found significantly higher protein and casein concentrations in DC milk than in GF milk. However, Law (1995) stated that there is less casein in goat's milk than in bovine milk.

The whey proteins in goat's and cow's milk are mainly composed of β-lactoglobulin (β-Lg) and α-lactalbumin (α-La). In contrast to bovine milk, the chromatographic pattern of DC and GW milk showed only one peak for β-Lg, so that for caprine milk no differentiation between β-Lg A and β-Lg B was made. Noni *et al.* (1996) also detected only one β-Lg fraction in goat's milk. In summarizing the genetic variants of β-Lg A and β-Lg B of bovine milk, there is less β-Lg in DC and GW milk compared to bovine milk which is in accordance to the results of Storry *et al.* (1983) and Law (1995). In accordance with results of Law (1995), the contents of α-La were almost comparable between the two goat breeds, whereas bovine milk had the least content.

5.3.2 Rennet Coagulation Properties

Regarding Table 5.2, RCT of DC milk was only slightly extended compared to the RCT of GW milk and was approximately 4 min lower than reported by Dimassi *et al.* (2005). One reason for the different result may be the sample preparation. In our experiments, pH_{20} was adjusted to 6.5 and 0.02 % (w/w) $CaCl_2$ was added to the different milks. The former strongly influences chymosin activity and the latter the aggregation of the para-casein micelles. In consequence, low pH values and the addition of $CaCl_2$ shorten RCT (Nájera *et al.* 2003). According to Clark and Sherbon (2000), RCT is delayed in milk with high protein content. This seems not to be valid for DC milk, which has a protein content of 3.5 %, similar to bovine milk (3.4 %). In contrast to DC milk, RCT of bovine milk was significantly delayed. Whey proteins only interact with casein and are included in the curd when they have been denaturated (Vasbinder *et al.* 2003). Hence, native whey proteins should not influence the coagulation process. However, Storry *et al.* (1983) found an increase in RCT in bovine milk when the concentration of α-La and serum albumin was increased. The effect of β-Lg content was not discussed, but the differing contents of native whey proteins in the different milks, shown in Table 5.1, may be a possible reason for variations in RCT. Further investigations should be carried out to elucidate this relationship.

Table 5.2: Rheological characteristics of skimmed bovine, Dahlem Cashmere and German White goat's milk adjusted to pH 6.50 and 30 °C (Mean ± S.D.).

	Bovine	Dahlem Cashmere	German White	n
F-60 ([N)	$(0.69 \pm 0.06)^a$	$(0.72 \pm 0.06)^a$	$(0.30 \pm 0.02)^b$	15
RCT (min)	$(16.4 \pm 1.2)^a$	$(12.0 \pm 1.6)^b$	$(10.5 \pm 1.0)^b$	6
AR (Pa/s)	$(0.054 \pm 0.006)^a$	$(0.115 \pm 0.004)^b$	$(0.049 \pm 0.003)^a$	6

^{a,b} means within a row with different superscripts differ ($P < 0.05$); AR: aggregation rate; F-60: maximum resistance force after 60 min of coagulation; n: number of samples; RCT: rennet coagulation time; S.D.: standard deviation

Figure 5.1 displays the increase in storage modulus G' depending on time and the origin of the milk. DC milk and GW milk showed a similar sigmoidal progression, whereas bovine milk differed evidently. The kinetic parameters G'_∞ and τ were obtained by fitting the experimental data (eq. 5.1). The model fits very well ($r^2 > 0.99$). G'_∞ significantly differed between DC (225 Pa) and GW milk (94 Pa), and between

GW (94 Pa) and bovine milk (215 Pa). Values for τ of DC and GW milk were comparable (~ 800 s).

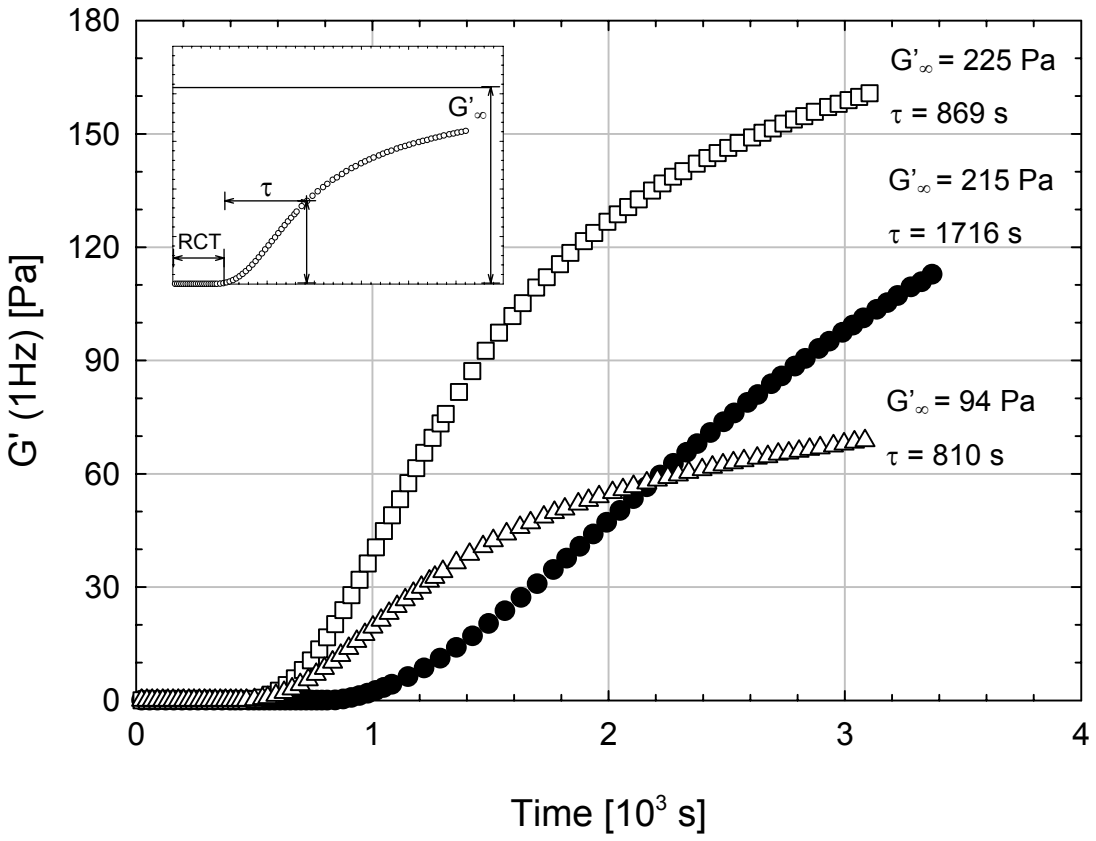

Figure 5.1: Storage modulus G' of bovine (●), DC (□) and GW (Δ) milk during gel formation at 30 °C. Kinetic parameters, G'_∞ and τ, were calculated according to equation 5.1 (the small picture shows the determination of the kinetic parameters).

High contents of α_{S1}-casein correlate with improved coagulation properties (Clark and Sherbon 2000), whereas milk with high β-casein content has poor coagulation properties (St-Gelais and Haché 2005). Goat's milk is usually poor in α_{S1}-casein, e.g. Law (1995) determined a content of 0.39 % compared to 0.8 % in bovine milk. Since recent findings (Dimassi *et al.* 2005) pointed out that DC milk had a high α_{S1}-casein content (0.64 %), the high curd firmness expressed as G'_∞ (Figure 5.1) and F-60 (Table 5.2), respectively, may be related to total casein concentration and to α_{S1}-casein content. Furthermore, goat milk with high levels of α_{S1}-casein possesses a desirable faster coagulation rate, leading to a firmer curd (Ambrosoli *et al.* 1988). Consequently, DC milk possessed the highest AR-value, resulting in the highest curd firmness (F-60) after 60 min of coagulation, followed by bovine milk. Hence, least

time is required to reach the appropriate rheological properties at which the cutting of the curd should be performed if using DC milk.

However, curd firmness is not only determined by the AR-value but also by the casein content as the comparison of GW and bovine milk illustrates. Although AR-values of bovine and GW goat's milk were comparable (Table 5.2), curd firmness differed significantly after 60 min of coagulation (F-60). As shown in Figure 5.1, G' of bovine milk increased during the measurement almost linearly with time, whereas the increase of G' of GW goat's milk attenuated markedly 900 s after RCT. It is suggested that this was due to a decrease in the number of bonds formed with time because of the lower casein content of GW milk compared to bovine milk (Table 5.1).

5.3.3 Kinetics of Syneresis

Figure 5.2 exemplarily shows the relative whey removal of bovine, DC and GW curd grains with an edge length of 11 mm as a function of incubation time at 40 °C.

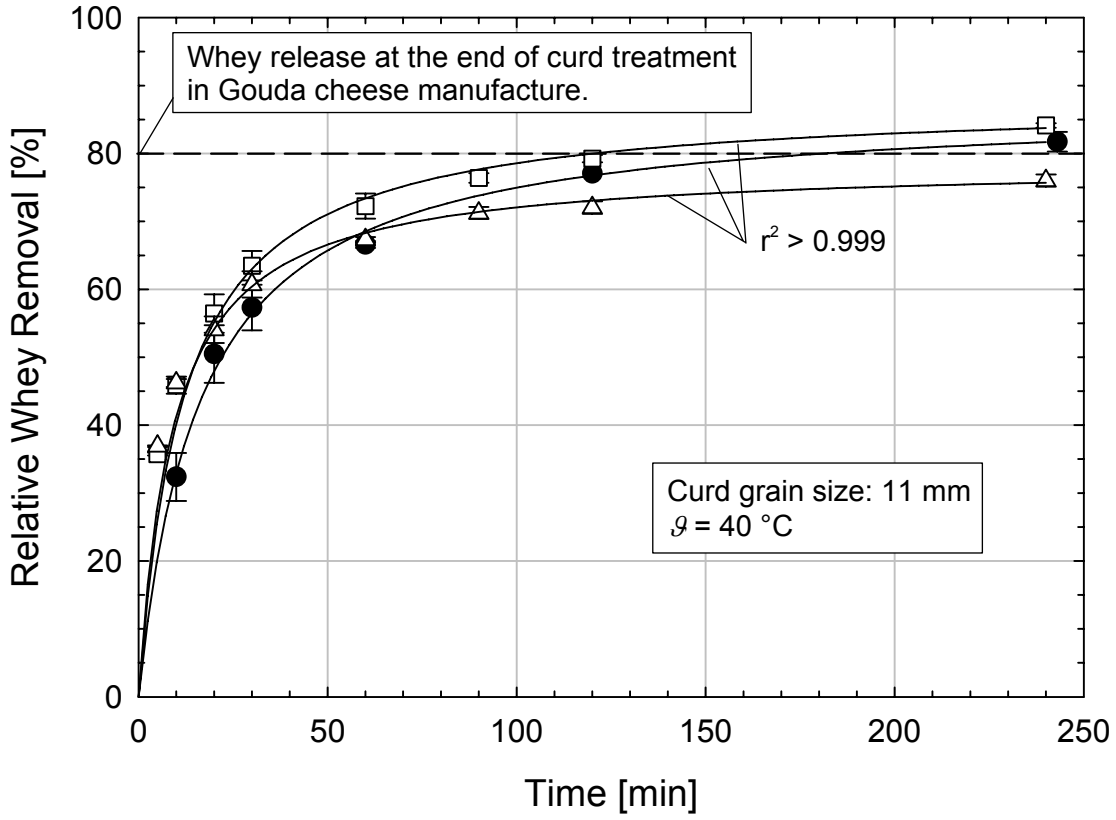

Figure 5.2: Relative whey removal (RWR) of bovine (•), DC (□) and GW (∆) curd grains with an edge length of 11 mm at 40 °C. Plotted are the mean values of four measurements with standard deviation and the calculated lines according to parameters obtained from equation 5.3 (Table 5.3).

The curvilinear progression of syneresis is similar for the three milks. It is obvious that within the first 30 min most of the serum of the curd grains was already expelled and that the rate of syneresis of curd grains from GW and DC milk was higher than the rate of grains from bovine milk. This is in accordance with results of Storry *et al.* (1983). The endpoint of syneresis, indicated by the asymptotic lapse of the curve, follow the pattern DC ≥ Bovine > GW. Within the first 20 min, syneresis of DC and GW curd grains was comparable, whereby between 20 and 240 min syneresis of DC and bovine curd grains was comparable.

Table 5.3 and 5.4 represent the kinetic parameters calculated by means of equation 5.3 for rennet curd grains with an edge length of 11 and 22 mm.

Table 5.3: Kinetic parameters of syneresis of the curd grains with an edge length of 11 mm (Mean ± S.E.).

Temp. (°C)	RWR_{max} (%)			$t_{1/2}$ (min)		
	Bovine (n ≥ 21)	DC (n ≥ 30)	GW (n ≥ 21)	Bovine (n ≥ 21)	DC (n ≥ 30)	GW (n ≥ 21)
25	$(48.7\pm1.9)^{a}$	$(46.5\pm1.4)^{a}$	$(53.5\pm1.2)^{a}$	$(33.6\pm5.2)^{A}$	$(23.0\pm2.9)^{B}$	$(16.4\pm2.6)^{C}$
30	$(70.1\pm1.6)^{a}$	$(64.8\pm1.0)^{b}$	$(57.8\pm1.1)^{c}$	$(31.5\pm3.0)^{A}$	$(20.1\pm1.8)^{B}$	$(9.6\pm1.2)^{C}$
35	$(82.2\pm1.0)^{a}$	$(81.6\pm0.9)^{a}$	$(74.4\pm0.7)^{b}$	$(18.1\pm1.4)^{A}$	$(17.1\pm1.3)^{A}$	$(12.2\pm0.9)^{B}$
40	$(87.2\pm0.6)^{a}$	$(87.9\pm0.7)^{a}$	$(78.5\pm0.5)^{b}$	$(16.4\pm1.1)^{A}$	$(11.8\pm1.1)^{B}$	$(9.0\pm0.9)^{C}$
50	$(89.4\pm0.5)^{a}$	$(90.3\pm0.2)^{a}$	$(81.3\pm0.4)^{b}$	$(6.1\pm0.5)^{A}$	$(5.0\pm0.2)^{B}$	$(4.8\pm0.5)^{B}$
60	$(88.7\pm0.3)^{a}$	$(89.5\pm0.2)^{a}$	$(81.3\pm0.3)^{b}$	$(4.1\pm0.3)^{A}$	$(3.3\pm0.2)^{B}$	$(3.3\pm0.4)^{B}$

^{a,b} means of RWR_{max} within a row with different superscripts differ ($P < 0.05$); ^{A,B} means of $t_{1/2}$ within a row with different superscripts differ ($P < 0.05$); DC: Dahlem Cashmere; GW: German White; n: number of measurements; RWR_{max}: relative whey removal after infinite time; S.E.: standard error; $t_{1/2}$: time that half of RWRmax is expelled

Table 5.4: Kinetic parameters of syneresis of the curd grains with an edge length of 22 mm (Mean ± S.E.).

Temp. (°C)	RWR_{max}			$t_{1/2}$		
	Bovine (n ≥ 21)	DC (n ≥ 31)	GW (n ≥ 31)	Bovine (n ≥ 21)	DC (n ≥ 31)	GW (n ≥ 31)
25	$(42.3 \pm 2.2)^{a,b}$	$(41.1 \pm 1.1)^{a}$	$(43.7 \pm 1.3)^{b}$	$(40.5 \pm 7.0)^{A}$	$(22.4 \pm 3.2)^{B}$	$(23.6 \pm 3.5)^{B}$
30	$(63.4 \pm 3.4)^{a}$	$(62.8 \pm 1.4)^{a}$	$(60.9 \pm 1.7)^{a}$	$(46.6 \pm 7.5)^{A}$	$(27.8 \pm 2.7)^{B}$	$(28.3 \pm 3.3)^{B}$
35	$(77.5 \pm 1.2)^{a}$	$(78.0 \pm 1.2)^{a}$	$(70.3 \pm 1.5)^{b}$	$(27.7 \pm 2.0)^{A}$	$(23.4 \pm 1.9)^{A}$	$(23.7 \pm 2.5)^{A}$
40	$(80.9 \pm 1.5)^{a}$	$(86.4 \pm 0.6)^{b}$	$(70.0 \pm 0.7)^{c}$	$(22.8 \pm 2.1)^{A}$	$(17.6 \pm 0.8)^{B}$	$(13.9 \pm 1.0)^{C}$
50	$(85.4 \pm 0.8)^{a}$	$(86.3 \pm 0.3)^{a}$	$(74.8 \pm 0.5)^{b}$	$(11.7 \pm 1.1)^{A}$	$(8.0 \pm 0.4)^{B}$	$(9.1 \pm 0.7)^{B}$
60	$(85.1 \pm 0.5)^{a}$	$(86.1 \pm 0.4)^{a}$	$(71.9 \pm 0.4)^{b}$	$(7.2 \pm 0.6)^{A}$	$(5.9 \pm 0.4)^{B}$	$(5.3 \pm 0.6)^{B}$

^{a,b} means of RWR_{max} within a row with different superscripts differ ($P < 0.05$); ^{A,B} means of $t_{1/2}$ within a row with different superscripts differ ($P < 0.05$); DC: Dahlem Cashmere; GW: German White; n: number of measurements; RWR_{max}: relative whey removal after infinite time; S.E.: standard error; $t_{1/2}$: time that half of RWRmax is expelled

For both grain sizes and independent of the milk origin, RWR_{max} increased and $t_{1/2}$ decreased with rising temperature. Hence, applying high temperatures during cheesemaking enhances syneresis as found by Walstra *et al.* (1985) and Calvo and Balcones (2000). Due to a higher surface to volume ratio, syneresis, at the same temperature, of grains with an edge length of 11 mm was accelerated compared to the larger grains (22 mm), leading to lower values for $t_{1/2}$ in Table 5.3. This is consistent with the work of Walstra *et al.* (1985) and Renault *et al.* (1997) who reported that syneresis is proportional to the surface area of curd particles.

Storry *et al.* (1983) and Calvo and Espinoza (1999) reported significant differences in the rates of syneresis between cow's, ewe's and goat's milk. Since $t_{1/2}$ in Table 5.3 and 5.4 was a measure of the rate of syneresis, this shows that the curd grains made from caprine milk released whey most rapidly. Values of $t_{1/2}$ follow the pattern Bovine > DC > GW.

The comparison of RWR_{max} in Table 5.3 and 5.4 for curd grain sizes of 11 and 22 mm showed a significant difference between DC and GW milk, and between bovine and GW milk. No significant differences were found between bovine and DC milk. RWR_{max} for DC and bovine milk was in general higher than for GW milk, indicating that milk composition and rennet curd structure of these two milks may be quite similar. This was confirmed regarding the results presented in section 5.3.1 and 5.3.2.

The main cause of syneresis is considered to be the rearrangement of the network of para-casein micelles with ongoing time (Dejmek and Walstra 2004). In comparison to milk with low casein content, renneted milk with high casein content forms a more compact para-casein network with an increased number of bonds. In consequence, under comparable external conditions, a higher pressure is exerted by the para-casein network on the entrapped whey, increasing RWR and RWR_{max}, respectively. Results in our pilot plant showed that for Gouda cheese manufacture a whey release (RWR) of approximately 80 % should be achieved at the end of curd treatment. Considering Figure 5.2, it is obvious that at 40 °C this value was only achieved for bovine and DC goat's milk.

5.4 Conclusion

The main objective of this work was to propose a strategy to evaluate cheesemaking properties of milk from different goat breeds. Milk composition, rennet coagulation properties along with syneresis were investigated, representing cheesemaking properties of milk that strongly determine the potential for cheese production.

(i) The quantity and quality of casein in DC goat's milk generated rheological properties that are favourable for semi-hard cheese production.

(ii) The firmness of the coagulum (F-60 value) made from DC milk was at cutting even higher than the gel made from bovine milk, promising a sufficient stability of the curd grains against the mechanical forces applied during curd treatment in semi-hard cheese production.

(iii) The syneresis experiments revealed that DC and bovine milk were comparable regarding the values for RWR_{max}, and that $t_{1/2}$ of DC and GW milk, respectively, were of similar size. Consequently, curd grains made from DC milk rapidly released whey during curd treatment, leading to a shorter curd treatment time with regard to a whey release of approximately 80 % that is typical in Gouda cheese manufacture. In contrast to curd grains made from GW milk, more whey was released with time leading to a high dry matter content in the curd grain.

In summary, the presented strategy was suitable to demonstrate the superior cheesemaking properties of DC goat's milk compared to GW goat's milk.

5.5 Acknowledgments

The authors would like to express their gratitude to the Research Station Oberer Lindenhof (University of Hohenheim) for providing the raw material for the experiments. The authors would also like to thank Dr. Dimassi for his support and discussion as well as Mr. Mertz and Mr. Migliore for their technical assistance and their valuable advice.

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6 THE IMPACT OF HOMOGENIZATION AND MICROFILTRATION ON RENNET-INDUCED GEL FORMATION

Abstract

The effects of the independent variables, homogenization pressure (p_1), concentration factor of microfiltration (i) and pH on the rheological properties of milk gels were studied. Non-destructive, oscillatory rheometry was used to determine rennet coagulation time (RCT), curd firming rate (CFR) and cutting time (CT). A two-level factorial, central composite design, considering two levels of i (1 and 2), pH (6.4 and 6.6) and p_1 (0 and 8 MPa), was applied. Second-order polynomial models successfully predicted ($R^2 > 0.92$) the relationship between processing parameters and rheological properties of the gels. pH had the most important influence on RCT, while CFR and CT were strongly influenced by i, pH and the interaction of i and pH. Results of texture analysis confirmed these observations, whereby an increase in p_1 strongly decreased gel firmness. This was not observed using rheometry, so that CT prediction for homogenized milk turned out to be difficult. It is therefore to be assumed, that in case of homogenization not the number of bonds at CT determines the firmness of the gel, but the mechanical properties of fat that is integrated into the original casein network due to its secondary milk fat globule membrane.

Keywords: *Cheese; Curd Firming Rate; Cutting Time; Homogenization; Microfiltration; Oscillatory Rheometry; pH; Rennet Coagulation Time; Texture*

Thomann, S., Schenkel, P. and Hinrichs, J. (2006). Impact of homogenization and microfiltration on rennet-induced gel formation. Journal of Texture Studies (submitted).

6.1 Introduction

Recent developments in microfiltration (MF) processes offer new opportunities for cheesemaking (Saboya and Maubois 2000). Up to 80 % of the throughput of existing cheese manufacturing facilities may be increased by applying MF of milk prior to cheesemaking (Thomet *et al.* 2004). Unlike ultrafiltration (UF), textural and flavour defects in cheeses are not expected since the use of MF retentate leads to composition very similar to that of conventional cheese curd (Papadatos *et al.* 2003). Results obtained using UF retentates for Cheddar cheesemaking indicate that the rapid curd firming rates (CFR) at milk protein levels above 4 % caused tearing of the relatively heavy textured curds before cutting was complete (Guinee *et al.* 1994). Furthermore, the authors reported excessive fat losses in the whey with increasing protein levels, reducing markedly cheese yield. Therefore, the determination of cutting time is an important monitoring step if MF retentates are used in cheese manufacture.

Homogenization of milk has been successfully applied in the manufacture of Cheddar (Emmons *et al.* 1980), cottage, Kachkaval, Mozzarella (Rowney *et al.* 2003) and Roquefort cheeses. Still, its implementation in cheesemaking is in particular limited to soft cheese manufacture. A decrease in cheese yield due to weak coagulum firmness and organoleptic problems in the ripened cheese are the discussed reasons. Jana and Upadhyay (1992) gave an excellent overview of the effect of homogenization on cheesemaking. Since homogenized fat globules are incorporated into in the casein network, the transfer of fat from milk into the cheese matrix may be increased leading to higher cheese yield.

Both pre-treatment technologies strongly alter the rennet coagulation properties of milk. After rennet addition, two major kinetic processes are involved in milk coagulation (Dalgleish 1979). The first step consists of the proteolysis of micellar κ-casein by rennet. The second one consists of the gel formation by aggregation of the destabilized casein micelles. Several authors consider three or more stages during milk coagulation as is reported by Castillo *et al.* (2003). It is to be emphasized, that in the present work two stages of milk coagulation were analyzed using non-destructive rheometry: rennet coagulation time (RCT) and CFR after occurrence of rennet coagulation. Besides the promising infrared technique (Payne *et al.* 1993; Castillo *et al.* 2003), rheometry is a useful measurement for monitoring milk coagulation. López

et al. (1998) found a linear correlation between the cutting time (CT) and the time of the maximum rate of increase of the storage modulus (G'). Guinee *et al.* (1994) determined CT by defining a fixed gel strength of G' = 16 Pa that was measured for each cheese milk in the rheometer.

Several studies evaluate the individual effects of homogenization, MF and pH on RCT and curd firming (Dalgleish 1980; Humbert *et al.* 1980; Robson *et al.* 1984; Ghosh *et al.* 1994; Caron *et al.* 1997; López *et al.* 1998; Nájera *et al.* 2003). Green *et al.* (1983) studied the influence of UF in a plant causing some homogenization of the fat on the structure and properties of rennet curd. The composition of Cheddar cheese made from homogenized milk prior to UF was improved, because of increased fat and moisture retention. Since homogenization and MF are both technologies that may enhance cheesemaking, it is surprising that little is known about their interaction and their influence on the rennet coagulation properties.

The main objective of this work was to study the interrelated effects of homogenization pressure (p_1), concentration factor of MF (i) and pH on RCT, CFR and CT. It is to be expected that the combination of MF and homogenization may decrease processing time in cheese manufacture, and reduce negative side-effects like high or low curd firmness at cutting.

6.2 Materials and Methods

6.2.1 Raw Material

Whole raw bovine milk was obtained on the first day of processing from the Research Station Meiereihof (University of Hohenheim, Germany). The ratio of fat to protein in the milk was adjusted to 0.9 giving average contents of (2.99 ± 0.14) % for fat, (3.32 ± 0.20) % for protein and (11.53 ± 0.35) % for dry matter.

6.2.2 Milk Processing

The standardized raw milk was immediately processed after pasteurization to produce four types of milk for experimental purposes (Figure 6.1): untreated milk, homogenized and unconcentrated milk, unhomogenized MF concentrated milk and

homogenized MF concentrated milk. Untreated milk, adjusted to pH 6.6 is in the following referred to as standard milk.

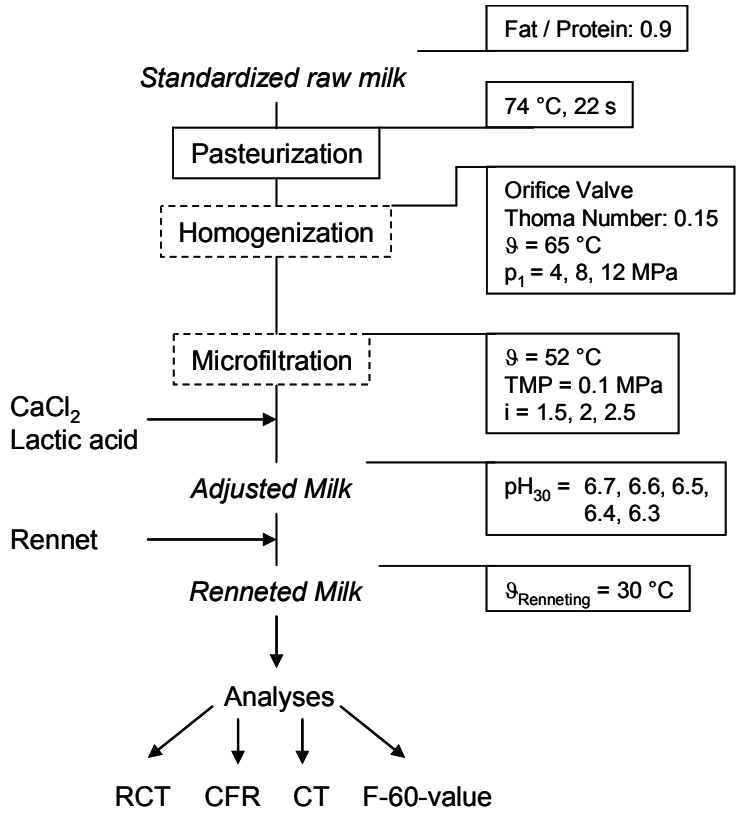

Figure 6.1: Processing of raw standardized milk. [ ]: optional; CFR: curd firming rate; CT: cutting time; F-60-value: curd firmness after 60 min of coagulation; RCT: rennet coagulation time; TMP: transmembrane pressure

Pasteurization (74 °C for 22 s) was carried out in a pilot-scale heating plant (Asepto, Dinkelscherben, Germany).

Homogenization was carried out prior to MF at 65 °C applying pressures (p_1) up to 12 MPa using an orifice valve provided by the University of Karlsruhe that was built into the cooling section of the heating plant. Back pressure (p_2) was realized using a needle valve. The ratio of p_2 to p_1 (Thoma Number) was adjusted to 0.15. Two oblique boreholes of diameter 0.5 mm are arranged in the centre of the orifice valve. The pitch α of the boreholes was 30°, so that two liquid jets collide behind the boreholes in an angle of 60°. The principle of this homogenization technique is described by Freudig *et al.* (2004).

MF concentration up to 2.5-fold was achieved using a pilot-scale membrane processing unit (model TFF, Pall SeitzSchenk, Waldstetten, Germany) equipped with

a ceramic Membralox gradient of permeability (GP) membrane (Type 7P-1940GP, Pall Exekia, Bazet, France) having an average pore size distribution of 0.1 µm and a total membrane area of 1.69 m^2. Prior to filtration, the milk was warmed up to 50 °C for at least 20 min and MF concentration was carried out afterwards at a temperature of (52 ± 2) °C and a transmembrane pressure of 10^5 Pa. The pressure drop along the membrane was $2 \cdot 10^5$ Pa giving a wall shear stress of 199 Pa. The concentration factor of MF (i) was calculated by the ratio of fat content in the retentate to fat content in the milk:

$$i = \frac{Fat_{Retentate}\ (\%)}{Fat_{Milk}\ (\%)} \tag{6.1}$$

After the individual treatment, milk and retentate, respectively, were immediately cooled down and stored in a cooling chamber at 6 °C until further treatment.

6.2.3 Chemical Analyses

The dry matter was determined at 90 °C using an infrared dryer (Moisture Analyzer MA30, Sartorius, Göttingen, Germany). Based on the Dumas method DIN 10467, total nitrogen was determined using a Leco FP-528 (Leco Instrumente GmbH, Mönchengladbach, Germany). Total protein was calculated by multiplying the nitrogen content with the milk specific factor of 6.38. The individual native whey protein fractions were measured by Reverse Phase - High Performance Liquid Chromatography according to the IDF (1996). The casein content was calculated according to Kersten (2001). The fat content was measured by the Gerber standard method (VDLUFA, C 15.3.2, 2003).

All analyses were at least performed in duplicate.

6.2.4 Size Distribution

Particle size distribution of milk samples was determined at 40 °C by static laser light scattering using a Coulter apparatus (LS230, Beckmann Coulter, Krefeld, Germany). The refractive indices were taken from Hinrichs (1994). The Polarisation Intensity Differential Scattering (PIDS) technology was activated, allowing the measurement of particles with diameters down to 40 nm. Measurements were made on each sample after dilution (1:1 vol.) with a casein dissociating medium (0.035 M EDTA/NaOH, pH 7.0 buffer).

6.2.5 Sample Preparation and Coagulation

For the rheological assays, 0.1 % (v/w) of 1:4 diluted calcium chloride solution (1.02382, VWR, Darmstadt, Germany) was added to milk and retentate, respectively. The sample was then warmed up to 30 °C and pH_{30} was adjusted with lactic acid (1.00366.2500, VWR, Darmstadt, Germany) or 1 M NaOH at 30 °C (pH_{30}: calibration of the pH-electrode was performed at 30 °C and the measurement was done without temperature correction). Each sample was equilibrated at 30 °C for 30 min in a water bath. For coagulation, 0.4 % (v/w) of 1:19 diluted rennet (ChyMax Plus, 190 IMCU/mL, Chymosin ≥ 99.9 %, Chr. Hansen, Horsholm, Denmark) was added to 30 mL of sample, mixed thoroughly for 1 min and transferred immediately to the rheometer geometry. For texture analysis, the sample was adjusted to pH_{30} 6.5 and was kept after rennet addition for 60 min at 30 °C in a water bath.

6.2.6 Texture Properties

Non-destructive and destructive measurements were carried out to characterize the coagulation properties of the individual milk sample (non-destructive) and the texture of the gels after 60 min of coagulation (destructive). Sample preparation was carried out as described before.

A Bohlin CS10 controlled-stress rheometer (Bohlin Instruments, Pforzheim, Germany) equipped with a double-gap device (DG40/50, Bohlin Instruments, Pforzheim, Germany) was used for studying the viscoelastic properties of the rennet-induced milk gels. After the addition of rennet, gel formation was monitored at 30 °C by measuring the storage modulus (G'), the loss modulus (G'') and the related phase angle δ = arc tan (G''/G') at fixed frequency (1 Hz). A strain amplitude of γ = 0.01 was applied, which is within the viscoelastic region of rennet-induced milk gels (Zoon *et al.* 1988). A solvent trap was used to avoid water loss and incrustation. RCT was deduced from the cross-over of the dynamic moduli (δ = 45°) according to Dimassi *et al.* (2005). CFR was determined from the slope at maximum increase of G'(t) according to Steffl *et al.* (1999). The G' value of the standard milk, which was untreated and adjusted to pH_{30} 6.6, was measured after 60 min of coagulation. This value was taken as reference for the determination of CT of the corresponding milk samples. The values of pH_{30}, coagulation temperature and CT of 60 min were

chosen for the standard because these are close to conventional semi-hard cheesemaking conditions. All experiments were performed in triplicate.

Texture analysis of the gels was performed at 30 °C according to Schreiber and Hinrichs (2000). 60 g of milk was used and the maximum resistance after 60 min of coagulation (F-60-value) was determined using a texture analyzer (Z2.5/TS1S, Zwick, Ulm, Germany) equipped with a load cell of 20 N. Each point was determined fivefold.

6.2.7 Experimental Design

The effects of the variables i, p_1 and pH on the rheological properties of milk gels were studied using a two-level factorial, central composite design (Kleppmann 2006). The design had 5 star points and 3 replicates of the center point. The 6th star point could not be realized because a negative homogenization pressure is not possible. This arrangement led to 17 treatments and each milk sample was measured threefold in the rheometer giving 51 results. The design was replicated twice. Since the first run of the design required 3 weeks, the design was divided in 3 blocks considering the weekly change in composition of the raw whole milk. According to Kleppmann (2006), one block should include the experimental star and the experimental cube may be subdivided into the remainder of the blocks. This was followed regarding the experimental design shown in Appendix 6.1.

6.2.8 Statistical Analysis

The STATGRAPHICS Plus package (version 5.1, Statistical Graphics Corp., Rockville, USA) was used for the statistical analysis. From the data obtained in the experimental design, an analysis of variance (ANOVA) was done to establish the presence or absence of significant differences in the coagulation parameters, considering i, p_1 and pH as factors. A second order polynomial equation was used to plot the response surface methodology (RSM) graphs.

6.3 Results

6.3.1 Milk and Concentrated Milk Composition

Ranges in composition of milk and concentrated milk are given in Appendix 6.2. The composition of the standardized milk was comparable within the survey period and MF concentration increased both, the casein and fat content.

6.3.2 Effects of Milk Processing and pH on Rennet-induced Coagulation

The effects of i, p_1, and pH on the rheological properties of milk gels were studied using a two-level factorial, central composite design. Experimental data were studied by means of ANOVA and the results (Table 6.1) showed that the effect of pH was highly significant ($P \leq 0.01$) for RCT, CFR and CT. The effect of i was also highly significant ($P \leq 0.01$) for the coagulation parameters, except RCT. p_1 had no significant effect on the parameters. CFR and CT were significantly affected by the two-factor (i x pH) interaction and CFR was significantly affected by (i x p_1). The quadratic terms for i and pH influenced RCT and CT at least significantly.

Table 6.1: Significance levels (P) of the analysis of variance for the effects of homogenization pressure (p_1), concentration factor of MF (i) and pH.

	i	p_1	pH	i^2	i x p_1	i x pH	p_1^2	p_1 x pH	pH^2	Blocks	LOF
RCT	*	NS	**	*	NS	NS	NS	NS	**	NS	NS
CFR	**	NS	**	NS	*	**	NS	NS	NS	NS	NS
CT	**	NS	**	**	NS	**	NS	NS	**	NS	NS

CFR: curd firming rate; CT: cutting time; LOF: lack of fit; NS: not significant; RCT: rennet coagulation time; * $P \leq 0.05$; ** $P \leq 0.01$

Table 6.2: Effects of homogenization pressure (p_1), concentration factor of MF (i) and pH on rennet coagulation properties; second-order polynomial equations with significant factors at $P \leq 0.05$

Equation		R^2	SE
RCT (min)	= 14.22 + 0.48 i - 3.97 pH + 0.82 $(i)^2$ + 1.08 $(pH)^2$	0.945	0.80
CFR (Pa/min)	= 10.95 + 8.46 i + 3.39 pH + 2.80 (i x pH) + 1.57 (i x p_1)	0.927	1.04
CT (min)	= 24.90 - 12.30 i - 7.93 pH + 7.04 $(i)^2$ + 2.63 (i x pH) + 1.68 $(pH)^2$	0.946	1.83

CFR: curd firming rate; CT: cutting time; RCT: rennet coagulation time; R^2: determination coefficient; SE: standard error of estimation

Second-order polynomial models for each dependent variable are given in Table 6.2. Since the P-values for the lack of fit test in the ANOVA table (Table 6.1) are greater or equal to 0.05, the models appear to be adequate for the observed data at the 95.0 % confidence level. The R^2 for the individual response was satisfying ($R^2 > 0.92$).

6.3.3 Impact of i and pH on CT

Figure 6.2 demonstrates the determination of CT by taking the G' value of the standard milk after 60 min of coagulation as reference. MF (i = 2) caused a strong increase of G' after clotting giving high CFR, so that the reference value for cutting was achieved earlier. The decrease in pH from 6.6 to 6.4 additionally shortened CT due to a decrease of RCT, so that aggregation and bond formation of the casein micelles started earlier. In addition, the CFR increased when pH was decreased, that is represented by a steeper slope (dG'/dt). RCT positively correlated ($P < 0.05$) with CT ($r = 0.51$) and CFR was negatively correlated ($P < 0.001$) with CT ($r = -0.72$), indicating the dependence between CT and RCT, and CFR, respectively.

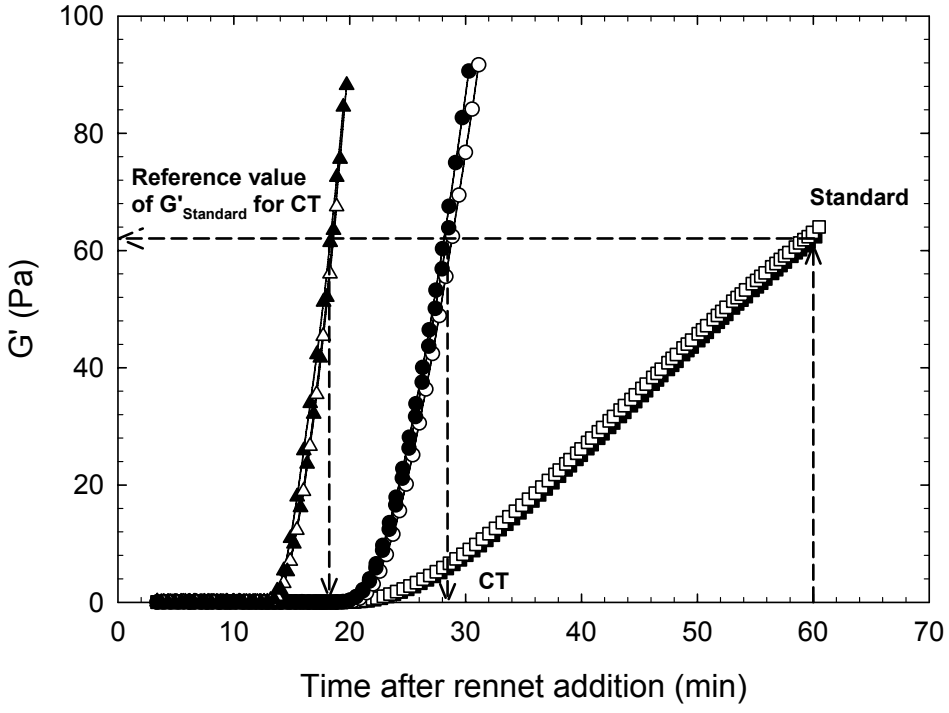

Figure 6.2: Determination of the cutting time (CT) of individual milk samples by taking the G' value of standard milk after 60 min of coagulation as reference. □, Standard (i = 1; 0 MPa; pH = 6.6); ●, i = 2; 0 MPa; pH = 6.6; Δ, i = 2; 0 MPa; pH = 6.4. Each treatment was measured threefold at 30 °C. The rennet coagulation time, which denotes the onset of gelation, is the time at which G' begins to increase.

The RSM graph for CT as a function of i and pH is shown in Figure 6.3. The CT value of the standard milk is additionally plotted to illustrate the significant influence of both variables on CT. CT decreased when i was increased and pH was decreased, respectively. CT of less concentrated milk systems decreased more compared to concentrated milk systems if pH was reduced. The interaction of pH 6.4 and i = 2 gave the lowest CT.

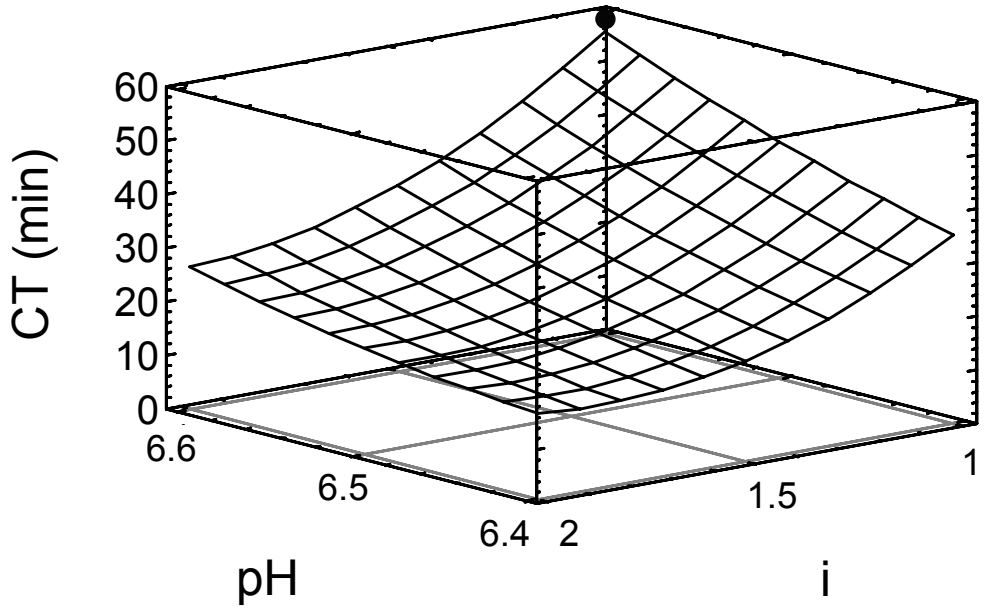

Figure 6.3: Response Surface Plot for the effect of concentration factor of MF (i) and pH on the cutting time (CT) of gels made from homogenized milk (4 MPa). ●, Standard (i = 1; 0 MPa; pH = 6.6)

6.3.4 Impact of p_1 on Rennet Coagulation Properties

According to Table 6.1 and 6.2, p_1 did not significantly influence the rennet coagulation properties of milk. However, the examination of the individual results generated some peculiarities that are presented in the following RSM graphs (Figure 6.4a - 6.4c). The corresponding value of the standard milk is additionally plotted in each graph. Figure 6.4a depicts the graph for RCT as a function of i and p_1. An increase in p_1 slightly decreased RCT, as is observed for values of i between 1 and 1.5. Above i = 1.5, RCT was slightly delayed. The RCT of the standard was approximately 4 min longer due to its higher pH of 6.6, demonstrating the influence of pH on RCT.

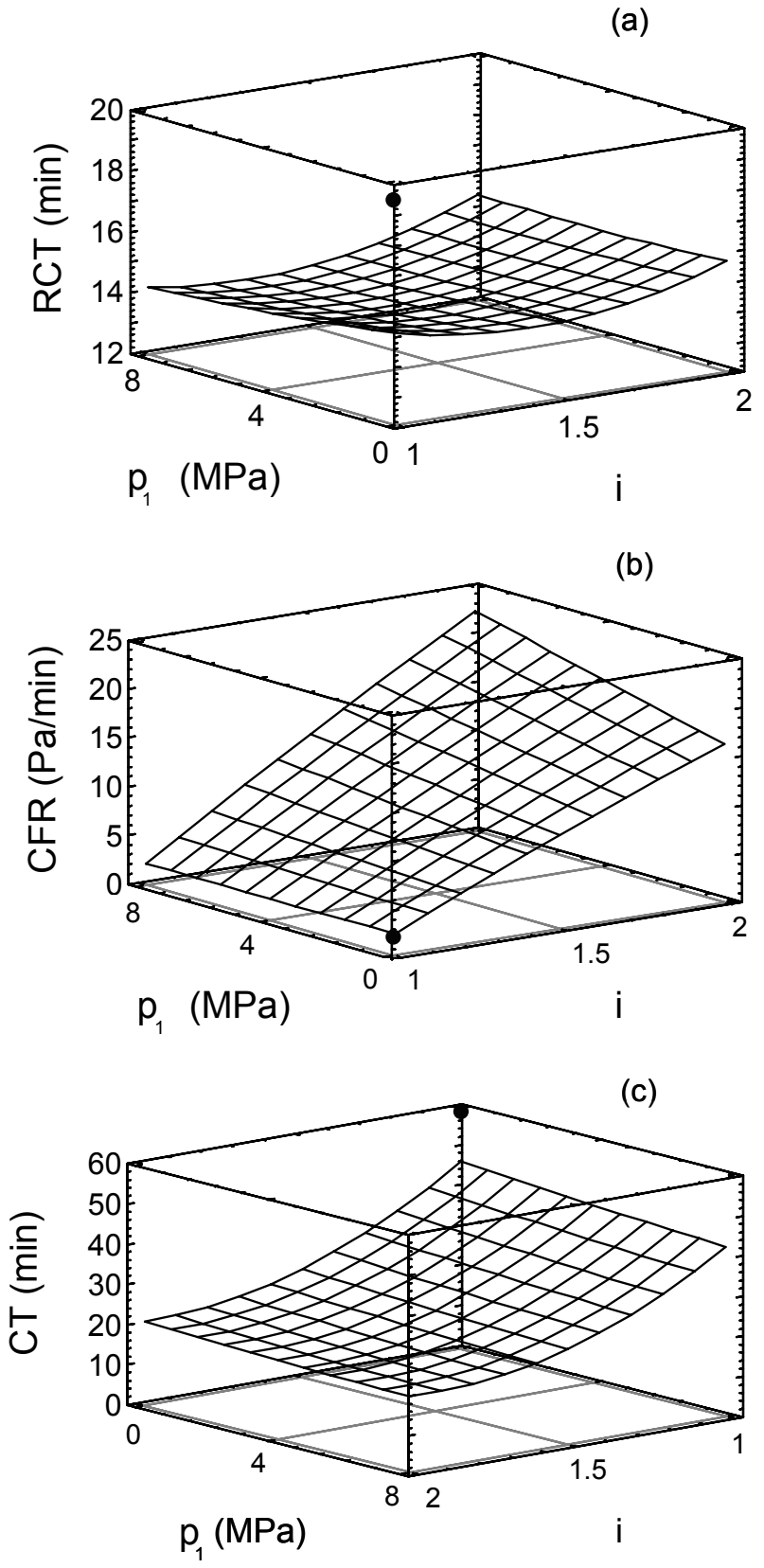

Figure. 6.4: Response Surface Plot for the effect of homogenization pressure (p_1) and concentration factor of MF (i) on (a) the rennet coagulation time (RCT), (b) curd firming rate (CFR) and (c) cutting time (CT) of gels made from milk adjusted to pH = 6.5. ●, Standard (i = 1; 0 MPa; pH = 6.6)

Figure 6.4b and 6.4c show the effect of i and p_1 on CFR and CT, respectively. CFR and CT were strongly affected when i was increased. With increasing homogenization pressures and contents of casein and fat, CFR markedly increased. The CFR value of homogenized, unconcentrated milk also increased, but the difference between unhomogenized and homogenized milk was with approximately 0.5 Pa/min too low to be identified in the RSM graph. CT slightly decreased in Figure 6.4c when p_1 was increased and this is consistent with the latter result. However, the firmness of the gels after 60 min of coagulation (F-60-value) decreased when p_1 was increased (Figure 6.5). From this result, a decrease in CFR and an increase in CT would be expected.

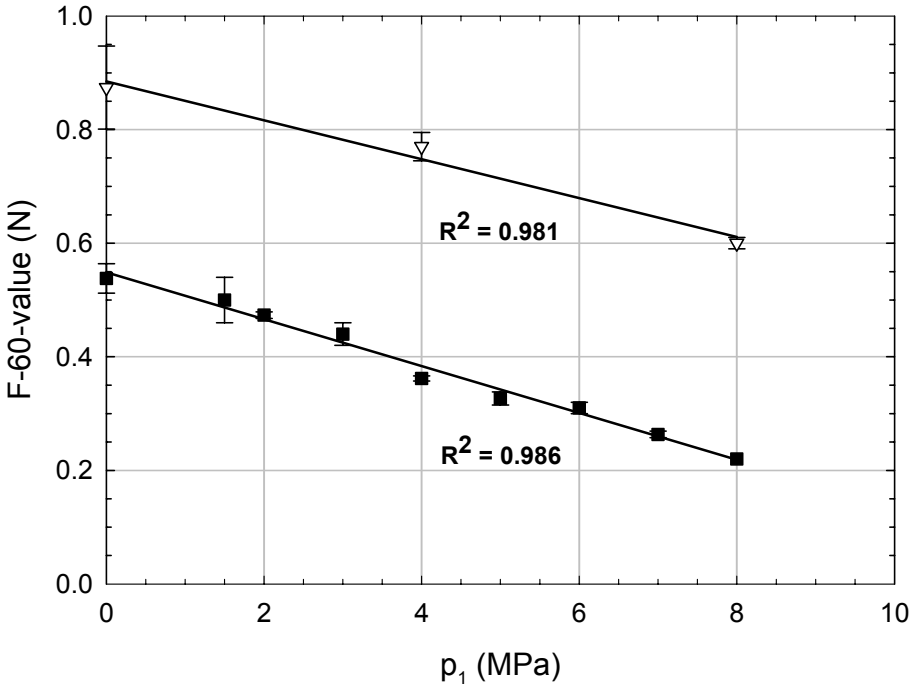

Figure 6.5: Firmness of rennet milk gels (pH = 6.5) after 60 min of coagulation (F-60-value) at 30 °C. Milk was homogenized at different pressures (p_1) prior to MF concentration. ■, unconcentrated milk (i = 1); ∇, MF concentrated milk (i = 1.44). Separate gels were made for each point. Values are mean of five measurements. Vertical bars indicate SD.

6.4 Discussion

The results obtained from the experimental design indicate that only the effect of pH on RCT was significant. RCT decreased when pH was decreased and this is consistent with results of van Hooydonk *et al.* (1986) and Nájera *et al.* (2003). It is

well known that chymosin possesses maximum milk clotting activity at pH values between 6 and 6.3. Consequently, a slight decrease in pH reduces RCT due to the enhanced activity of the enzyme.

The increase in casein and fat content due to MF did not significantly influence RCT (Table 6.1). This is in agreement with results of Dalgleish (1980) and Lucisano *et al.* (1985), who studied the effect of UF on RCT. Hence, an increase in casein and fat content due to MF up to i = 2 did not alter RCT, although the ratio of chymosin to casein decreased. Robson and Dalgleish (1984) and Hayes and Kelly (2003) stated that RCT was reduced when homogenization pressure was increased. In Figure 6.4a, a slight tendency of RCT reduction was also observed when p_1 was increased. Since this effect was more pronounced for unconcentrated milk compared to MF concentrates and was much lower compared to the effect of pH, RCT was therefore not significantly affected by p_1 (Table 6.1).

CT is significantly related to CFR ($r = -0.72$, $P < 0.001$) and to RCT ($r = 0.51$, $P < 0.05$), demonstrating the dependence between CT and CFR, and RCT, respectively. If pH was decreased, CT significantly decreased (Figure 6.3) and this can be due to a reduced RCT and an additionally increased CFR. This supports the findings of López *et al.* (1998) and Steffl *et al.* (1999), and the background of the observed effect of pH is in detail discussed by van Hooydonk *et al.* (1986) and Mishra *et al.* (2005). In consequence, a decrease in pH accelerates RCT and increases CFR, so that cutting in cheese manufacture can be performed earlier.

The increase of casein due to MF significantly affected CT due to a higher CFR (Figure 6.2). MF increased the number of structure-forming particles and after coagulation more bonds per time are linked within the network giving higher G' and CFR values. This is consistent with results of Caron *et al.* (1997) and Mishra *et al.* (2005). Guinee *et al.* (1996) studied the effect of different heat treatments on the rennet coagulation properties of retentates obtained by UF. An increase in protein concentration due to UF of pasteurized milk increased CFR values. As MF and UF increases the casein content in milk, this is to be expected. Our results show that CT was reduced to more than 50 % if milk was concentrated 2-fold, shortening tremendously processing time in cheese manufacture. This positive effect was even intensified if pH was additionally decreased (Figure 6.3).

Regarding Figure 6.5, a rise in casein content from i = 1 to i = 1.44 increased the F-60-values after 60 min of coagulation. The low coagulum firmness due to homogenization of unconcentrated milk was therefore compensated. Since RCT for concentrated and unconcentrated milk was not altered (Figure 6.4a), the increase in curd firmness of concentrated milk is related to its higher CFR, indicating the dependence of CFR and curd firmness of concentrated milk systems.

Despite the fact that p_1 did not significantly affect the rennet coagulation properties of milk, some peculiarities were noticed that may be disregarded if just taking the results in Table 6.1 and 6.2 into consideration. Since homogenization of milk reduces curd firmness with increasing pressures as presented in Figure 6.5 and reported by Ghosh *et al.* (1994), a reduced CFR and a prolonged CT were expected. In summary, the results in Figure 6.4 reflect the opposite of what was expected. CFR of homogenized, unconcentrated milk was approximately 0.5 Pa/min higher compared to CFR of unhomogenized, unconcentrated milk. Therefore, CT in Figure 6.4c slightly decreased for unconcentrated milk with an increase in p_1. For homogenized and concentrated milk systems, CFR was even increased by the interaction of i and p_1 (Figure 6.4b). CT was not altered, since the effect of i dominates curd firming, so that the markedly influence of i and p_1 on CFR is of little consequence on CT.

Hayes and Kelly (2003) observed the same disparity, if comparing results obtained by rheometry and texture analysis. A drop in pH due to homogenization, as reported there, may be excluded, since pH in our experiments was adjusted before the individual experiment. The change in composition and structure of the milk fat globule membrane due to homogenization may partly explain this observation. Approximately 75 % of the secondary milk fat globule membrane, that replaces the native membrane after homogenization, is covered with casein (Walstra and Oortwijn 1982; Cano-Ruiz and Richter 1997). With increasing pressures, the average fat globule size is reduced (Appendix 6.2). The total fat globule surface area is increased up to 5- to 10-fold of the original value. Therefore, homogenized fat globules may behave to some extent like large casein micelles and participate in enzymic coagulation processes (Buchheim 1986). Since the area per κ-casein molecule is increased from 40 nm^2 for unhomogenized milk to 80 nm^2 for homogenized milk (Robson and Dalgleish 1984), a lower level of proteolysis is necessary to destabilize the casein-fat particles. Hence, homogenization increases the number of structure-forming particles

and furthermore reduces steric stabilization by decreasing the energy barrier to close approach. Consequently, the probability for aggregation of casein and casein-fat particles is higher compared to unhomogenized milk as steric repulsion is lower. Thus, faster bond formation appears that gives therefore higher CFR values if small non-destructive deformation measurement is applied. Furthermore, the volume of the network relative to that of the interstices increases, effectively reducing the ease of movement of the strands (Green *et al.* 1983).

Applying high deformation, like at cutting or in texture analysis, the homogenized fat globule acts different. Unlike an unhomogenized fat globule, the homogenized globule is incorporated into the network and if mechanical stress is applied, the casein-fat network may evade, lowering the resistance of the gel. Furthermore, pictures obtained by scanning electron microscopy showed that in comparison to untreated milk the protein-fat strands of microfluidized milk (very intensive homogenization) were bulky and of uneven thickness and apparently more strands ended in nodules that were not tied into the gel structure (Tosh and Dalgleish 1998). This observation may also explain the weak curd firmness of homogenized milk. It is to be assumed, that in case of homogenization not the number of bonds at CT determines the firmness of the gel, but the mechanical properties of fat that is integrated into the original casein network due to its secondary milk fat globule membrane.

6.5 Conclusion

The main objective of this study was to investigate the impact of homogenization, MF, pH and their interaction on rheological properties of rennet-induced milk gels. Non-destructive (rheometry) and destructive measurements (texture analysis) were carried out to characterize the coagulation properties.

(i) Only RCT was significantly influenced when pH was decreased because enzyme activity increased.

(ii) Determination of CT depending on pH, i and their interaction was possible using rheometry. CT significantly decreased when i was increased and/or pH decreased. A rise in i increased the CFR as the number of structure-forming

particles was increased. A reduction of pH decreased RCT due to a changed enzyme activity and increased the CFR as electrostatic repulsion is reduced.

(iii) For unconcentrated and homogenized milk, CT prediction turned out to be difficult considering the disparity in results obtained from rheometry and texture analysis. In the case of homogenization, curd firmness at CT is determined by the mechanical properties of fat that is integrated into the original casein network due to its secondary milk fat globule membrane.

(iv) Results obtained from texture analysis indicate that weak curd firmness due to homogenization may be compensated if MF was additionally applied.

(v) Less cheesemaking agents like $CaCl_2$ and rennet were used if concentrated milk was analyzed. However, RCT was not altered and CT tremendously decreased with increasing i. Hence, costs, material and time in cheesemaking can be saved by integrating MF technology.

Since the curd structure at cutting influences syneresis in cheesemaking, CT should be individually determined before further syneresis experiments are carried out. This was investigated elsewhere, and bringing together all these data provide useful information concerning the implementation of innovative technologies in conventional cheese manufacture.

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6.7 References

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6.8 Appendix

Appendix 6.1: Values of independent variables of each coded and actual values for the central composite design.

Treatment	Block	Coded values			Actual values		
		i	p_1	pH	i	p_1 (MPa)	pH
1 (Cube)	1	-1	-1	1	1	0	6.4
2 (Cube)	1	1	-1	1	2	0	6.4
3 (Cube)	1	-1	-1	-1	1	0	6.6
4 (Cube)	1	1	-1	-1	2	0	6.6
5 (Cube)	1	0	-1	0	1.5	0	6.5
6 (CP)	1	0	0	0	1.5	4	6.5
7 (Star)	2	-1.2	0	0	0.9	4	6.5
8 (Star)	2	0	0	-2	1.5	4	6.7
9 (Star)	2	0	0	2	1.5	4	6.3
10 (CP)	2	0	0	0	1.5	4	6.5
11 (Star)	2	2	0	0	2.5	4	6.5
12 (Star)	2	0	2	0	1.5	12	6.5
13 (Cube)	3	1	1	-1	2	8	6.6
14 (Cube)	3	1	1	1	2	8	6.4
15 (Cube)	3	-1	1	-1	1	8	6.6
16 (Cube)	3	-1	1	1	1	8	6.4
17 (CP)	3	0	0	0	1.5	4	6.5

CP: center point; i: concentration factor of MF; p_1: homogenization pressure

Appendix 6.2: Averaged contents of dry matter (DM), fat, total protein, whey protein (WP) and casein in milk and volume-to-surface mean diameter ($d_{3,2}$).

Treatment	DM (%)	Fat (%)	Protein (%)	WP (%)	Casein (%)	$d_{3,2}$ (µm)
1; 3	11.51	2.95	3.34	0.43	2.76	3.37
2; 4	16.82	5.85	5.92	0.44	5.33	n.d.
5	13.56	4.30	4.63	0.41	4.07	3.41
6	13.63	4.15	4.60	0.41	4.05	0.75
3	11.55	2.95	3.31	0.43	2.73	2.91
7	11.01	2.73	2.99	0.44	2.40	0.77
8; 9; 10	13.86	4.35	4.58	0.41	4.02	0.87
11	20.01	7.35	7.40	0.52	6.73	0.59
12	13.54	4.28	4.41	0.40	3.86	0.56
3	11.45	2.98	3.19	0.40	2.65	3.36
13; 14	16.65	5.75	5.78	0.46	5.17	0.65
15; 16	11.61	3.03	3.26	0.44	2.67	0.68
17	13.84	4.23	4.63	0.42	4.06	0.91

n.d.: not determined; values of DM and $d_{3,2}$ are means of three replicates; values of fat, protein and whey protein are means of two replicates

7 Effect of Homogenization, Microfiltration and pH on Curd Firmness and Syneresis of Curd Grains

Abstract

The impact of the independent variables, homogenization pressure (p_1), concentration factor of microfiltration (i) and pH on curd firmness (CF) and syneresis of curd grains was studied. Texture analysis was used to characterize CF of the rennet-induced gels. The analysis of a two-level factorial design revealed that i, p_1, pH and the interaction of i and pH had the most important influence on CF. Cutting time was therefore individually determined for each milk system using small amplitude oscillatory rheometry for generating comparable conditions for the syneresis experiments. Syneresis of curd grains with a diameter of 11 mm was followed at 35 °C close to semi-hard cheesemaking conditions. The permeate release during microfiltration was taken into consideration, allowing an evaluation of syneresis of grains made from concentrated and unconcentrated milk. It was shown that with increasing milk concentration less curd treatment time was needed to reach a certain syneresis value. Hence, total processing time in cheesemaking is decreased. Analysis of variance revealed that syneresis was affected by the individual variables. Kinetic parameters were satisfactorily estimated through regression ($R^2 > 0.98$) and it was shown that milk composition and concentration due to microfiltration markedly influenced the endpoint of syneresis, RWR_{max}. The experiments demonstrate that microfiltration and homogenization can be combined to reach CF and syneresis comparable to untreated milk used in conventional cheesemaking. This meets one claim of the cheese industry when implementing both technologies in the manufacture process, since consistency and quality of the ripened cheese are expected to be unchanged.

Keywords: *Curd Firmness; Homogenization; Kinetics; Microfiltration; Syneresis*

Thomann, S., Schenkel, P. and Hinrichs, J. (2008). Effect of homogenization, microfiltration and pH on curd firmness and syneresis of curd grains. LWT - Food Science and Technology *41*, 826-835.

7.1 Introduction

The effect of homogenization of milk has been studied in the manufacture of Cheddar (Emmons *et al.* 1980), cottage, Kachkaval, Mozzarella (Rowney *et al.* 2003) and Roquefort cheeses. Still, its implementation in cheesemaking is in particular limited to soft cheese manufacture. A weak coagulum firmness leading to curd shattering and increased losses of curd fines (Lemay *et al.* 1994), a retarded whey release during curd treatment and organoleptic problems in the ripened cheese are the discussed reasons. Jana and Upadhyay (1992) gave an excellent overview of the effect of homogenization on cheesemaking. Since homogenized fat globules are incorporated into the casein network, the transfer of fat from milk into the cheese matrix may be increased leading to higher cheese yield.

Recent developments in microfiltration (MF) processes offer new opportunities for cheesemaking (Saboya and Maubois 2000). Up to 80 % of the throughput of existing cheese manufacturing facilities may be increased by applying MF of milk prior to cheesemaking (Thomet *et al.* 2004). Textural and flavour defects in cheeses are not expected since the use of MF retentate leads to composition very similar to that of conventional cheese curd (Papadatos *et al.* 2003). However, results obtained from using ultrafiltration (UF) retentates for Cheddar cheesemaking indicate that protein levels above 4 % caused tearing of the relatively heavy textured curds before cutting was complete (Guinee *et al.* 1994). Furthermore, the authors reported excessive fat losses in the whey with increasing milk protein levels, reducing markedly cheese yield. Therefore, cutting time (CT) and syneresis are important monitoring steps if MF retentates are used in cheese manufacture.

Lelievre (1977) stated that curd firmness (CF) slightly influenced syneresis but the results did not show a clear tendency whether syneresis was increased with increasing CF or not. However, chemical analyses of whey indicated that the protein and fat content of the initial flux of liquid from gels decreased as CF increased. Marshall (1982) surveyed the effect of CF on the rate of whey expulsion and showed that the syneresis rates were higher from curds cut early and late than from those cut at intermediate times. Grundelius *et al.* (2000) studied the effect of the storage modulus (G') of curd on syneresis of a single curd grain and found a significant relation but gave no statement concerning an increase in syneresis with increasing values of G' and in reverse. Johnson, Chen and Jaeggi (2001) reported that a firmer

coagulum at cutting resulted in an increase in cheese moisture that may be attributed to a changed syneresis behaviour during curd treatment.

Besides the promising infrared technique developed in order to follow coagulation and predict the cutting time of renneted milk by using an on-line fibre optic sensor that measures diffuse reflectance (Castillo *et al.* 2003), rheometry is a useful measurement for assessing the rennet coagulation properties of cheese milk (Auldist *et al.* 2001). For generating equal firmness values at cutting of standardized UF retentates with protein levels ranging from 30 to 82 g/L, Guinee *et al.* (1994) determined the CT by defining a fixed gel strength of G' = 16 Pa that was measured for each milk in the rheometer. In the presented study, a similar method was applied to individually determine CT for each milk system using small amplitude oscillatory rheometry.

Several studies evaluate the individual effects of homogenization, protein concentration and pH on CF and syneresis (Humbert *et al.* 1980; Walstra *et al.* 1985; Casiraghi *et al.* 1987; Ghosh *et al.* 1994; Grundelius *et al.* 2000; Caron *et al.* 2001; Schreiber and Hinrichs 2000). Green *et al.* (1983) studied the influence of homogenization and UF on the structure and properties of rennet curd and Cheddar cheese. The composition of Cheddar cheese made from milk homogenized prior to or during UF was improved, because of increased fat and moisture retention. The improvement was attributed to the reduced hardness of the cheeses leading to a texture comparable to the control cheese. In contrast, cheese made exclusively from concentrated milk was more granular and drier than the control. Since homogenization may increase cheese yield (Metzger and Mistry 1994; Brito *et al.* 2006) and MF increases plant efficiency and decreases cost of cheese production (Papadatos *et al.* 2003), it is surprising that little is known about their interaction and their influence on CF and syneresis.

So, the main objective of the study was to investigate the interrelated effects of homogenization pressure (p_1), concentration factor of MF (i) and pH on CF and syneresis of curd grains. The casein content was increased by MF instead of the addition of casein powder since nowadays MF becomes widely accepted in the industry for milk processing and standardization. Three-dimensional syneresis of curd grains was followed under conditions close to semi-hard cheese manufacture. It is to be expected that the combination of MF and homogenization may reduce

negative side-effects of the individual technology like high or low CF at cutting which would cause loss of curd fines during the syneresis process and finally decrease cheese yield. A low firmness due to homogenization may be equalized due to MF that increases CF and vice versa.

7.2 Materials and Methods

7.2.1 Raw Material and Milk Processing

Over a period of three coherent weeks and on the first day of each experimental week, whole raw bovine milk was freshly obtained from the Research Station Meiereihof (University of Hohenheim, Germany). The ratio of fat to protein in the milk was adjusted to 0.90 $\pm$ 0.02 by adding the appropriate amount of skim milk to the raw milk. Within this period, the average composition of the milk was 29.6 $\pm$ 0.1 g kg^{-1} for fat, 33 $\pm$ 0.6 g kg^{-1} for protein and 114.9 $\pm$ 3.0 g kg^{-1} for dry matter (DM).

After pasteurization (74 °C for 22 s) in a pilot-scale heating plant (Asepto, Dinkelscherben, Germany) the standardized raw milk was immediately processed to produce four types of milk for experimental purposes (Figure 7.1): untreated milk, homogenized and unconcentrated milk, unhomogenized MF concentrated milk and homogenized MF concentrated milk. In the following, untreated milk, adjusted to pH 6.6 is referred to as standard milk.

Homogenization was carried out at 65 °C prior to MF, applying pressures (p_1) of 4, 8 and 12 MPa using an orifice valve provided by the University of Karlsruhe that was built into the cooling section of the heating plant. Back pressure (p_2) was realized using a needle valve. The ratio of p_2 to p_1 (Thoma Number) was adjusted to 0.15. Two oblique boreholes of diameter 0.5 mm are arranged in the centre of the orifice valve. The pitch α of the boreholes was 30°, so that two liquid jets collide behind the boreholes in an angle of 60°. The principle of this innovative homogenization technique is described by Freudig (2004).

MF concentration up to 2.5-fold was achieved using a pilot-scale membrane processing unit (model TFF, Pall SeitzSchenk, Waldstetten, Germany) equipped with a ceramic Membralox gradient of permeability (GP) membrane (Type 7P-1940GP,

Pall Exekia, Bazet, France) having an average pore size distribution of 0.1 µm and a total membrane area of 1.69 m^2. Prior to filtration, the milk was warmed up to 50 °C for at least 20 min and MF concentration was carried out afterwards at a temperature of (52 ± 2) °C and a transmembrane pressure of 10^5 Pa. The pressure drop along the membrane was $2 \cdot 10^5$ Pa giving a wall shear stress of about 199 Pa. The concentration factor of MF (i) was calculated by the ratio of fat content in the retentate to fat content in the milk. The value of i = 0.9 was adjusted by adding the appropriate amount of fresh MF permeate to the unconcentrated milk.

$$i = \frac{\text{Fat}_{\text{Retentate}}\ (\%)}{\text{Fat}_{\text{Milk}}\ (\%)} \tag{7.1}$$

For calculation of i of skim milk, the casein content was determined. After the individual treatment, milk and retentate, respectively, were immediately cooled down and stored in a cooling chamber at 6 °C until further treatment.

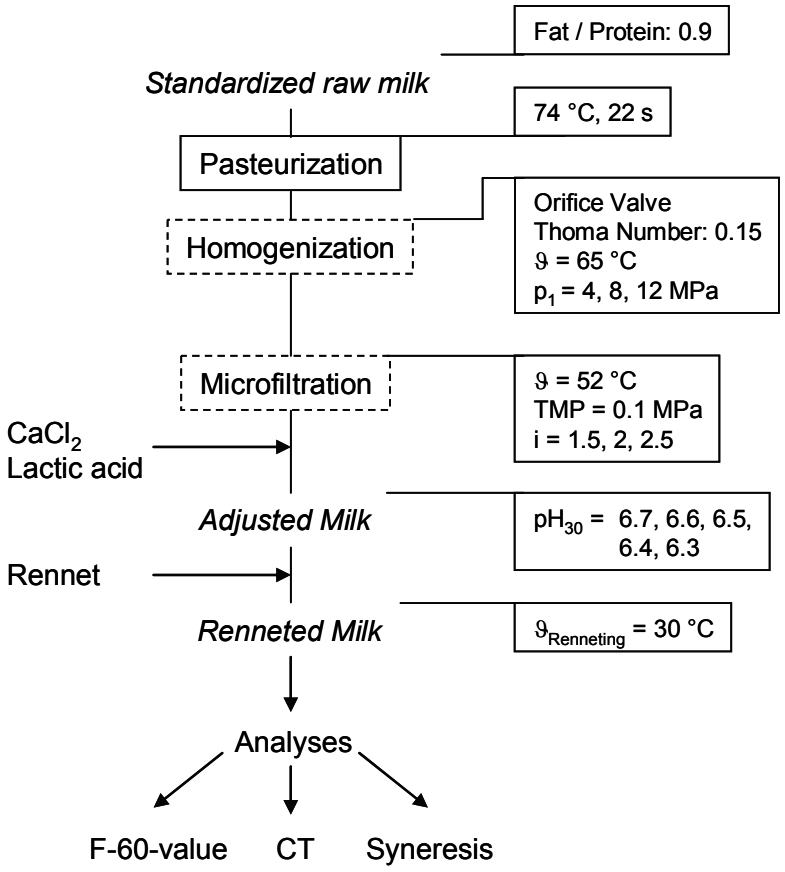

Figure 7.1: Processing of raw standardized milk. (: optional; CT: cutting time; F-60-value: curd firmness after 60 min of coagulation; i: concentration factor of MF; p_1: homogenization pressure; TMP: transmembrane pressure)

7.2.2 Size Distribution

Particle size distribution of milk samples was determined at 40 °C by laser light scattering using a Coulter apparatus (LS230, Beckmann Coulter, Krefeld, Germany). The refractive indices were taken from Hinrichs (1994). The Polarisation Intensity Differential Scattering (PIDS) technology was activated, allowing the measurement of particles with diameters down to 40 nm. Measurements were made on each sample after dilution (1:1 vol.) with a casein dissociating medium (0.035 M EDTA/NaOH, pH 7.0 buffer).

7.2.3 Sample Preparation and Coagulation

Calcium chloride (1.02382.1000, VWR, Darmstadt, Germany) was added in a concentration of 0.2 g kg^{-1} to milk and retentate. The sample was then warmed up to 30 °C and pH_{30} (pH_{30}: calibration of the pH-electrode was performed at 30 °C and the measurement was done without temperature correction) was adjusted with lactic acid (1.00366.2500, VWR, Darmstadt, Germany) or 1 M NaOH to the appropriate value. Each sample was equilibrated at 30 °C for at least 20 min in a water bath. For coagulation, 0.2 mL kg^{-1} chymosin (ChyMax Plus, 190 IMCU/mL, Chr. Hansen, Hoersholm, Denmark) was added to the sample, mixed thoroughly for 1 min and transferred immediately to the rheometer geometry. A separate series of samples for texture analysis was prepared as described before but kept after rennet addition for 60 min at 30 °C in a water bath. Likewise, samples for syneresis tests were kept separately after rennet addition at 30 °C in a water bath until cutting.

7.2.4 Texture Properties

A Bohlin CS10 controlled-stress rheometer (Bohlin Instruments, Pforzheim, Germany) equipped with a double-gap device (DG40/50, Bohlin Instruments, Pforzheim, Germany) was used for studying the viscoelastic properties of the rennet-induced milk gels. After the addition of rennet, gel formation was monitored at 30 °C by measuring the storage modulus (G'), the loss modulus (G'') and the related phase angle δ = arc tan (G''/G') at fixed frequency (1 Hz) and a strain amplitude of γ = 0.01. A solvent trap was used to avoid water loss and incrustation. The G' value of the standard milk, which was untreated and adjusted to pH 6.6, was measured after 60 min of coagulation. This value was taken as a reference for the determination of the CT of the other milk and retentate samples. pH 6.6, coagulation temperature of

30 °C and CT of 60 min were chosen for the standard because these values correspond to conditions frequently applied in semi-hard cheesemaking. All experiments were performed in triplicate.

Texture analyses of the gels were performed at 30 °C according to Schreiber *et al.* (2000). 60 g of milk was used and the maximum resistance after 60 min of coagulation (F-60-value) was determined using a texture analyzer (Z2.5/TS1S, Zwick, Ulm, Germany) equipped with a load cell of 20 N. A geometry of soldered crossed wiring (30 mm diameter) penetrated the rennet gel with a crosshead speed of 0.5 mm/s to a maximum depth of 15 mm. Each point was determined from five independent measurements.

7.2.5 Syneresis

Syneresis was followed with the Dynamic Model System (Huber *et al.* 2001). Centrifugal cups (DURHAN tube with screw thread, DIN 12216, VWR, Germany) were filled with 50 mL of MF permeate (55.8 g kg^{-1} for DM, pH 6.55) and were tempered at 35 °C in an incubator (WTR-1, Infors AG, Switzerland). MF permeate was freshly processed in each survey week by MF of the standardized cheese milk. Hence, curd grains and permeate were derived from the same milk source, generating conditions comparable to curd treatment applied in cheese manufacture. The coagulum was cut at the appropriate CT, individually determined for each milk system as previously described. Four curd grains with a diameter of 11 mm were transferred to the tempered centrifugal cups and curd treatment was simulated by shaking the cups and grains, respectively, with a frequency of 200 rpm and an amplitude of the swinging disk of 7 mm. Syneresis of rennet curd grains was measured after 10, 20, 30, 60, 90 and 240 min.

The relative whey release, RWR, indicating the time-dependent syneresis, was calculated from the initial weight of the grains, m_0, and the weight of the grains after treatment, m_t, according to equation 7.2.

$$RWR = \left(1 - \frac{m_t}{m_0}\right) \cdot 100 \qquad (7.2)$$

Since MF is a processing step that removes milk serum (permeate) prior to cheesemaking, this has to be considered in the calculation of syneresis of curd grains made from concentrated milk (eq. 7.3).

$$RWR = \left(1 - \frac{m_t}{i \cdot m_0}\right) \cdot 100 \tag{7.3}$$

Additionally, syneresis was followed by the determination of DM of the curd grains at 100 °C using an infrared dryer (MA 30, Sartorius, Göttingen, Germany).

Syneresis of each processed milk was determined from three independent measurements.

7.2.6 Modelling

Syneresis of curd grains made from unconcentrated milk was modelled by means of equation 7.4 according to Thomann *et al.* (2006).

$$\frac{t}{RWR} = \frac{1}{RWR_{max}} \cdot t + \frac{t_{1/2}}{RWR_{max}} \tag{7.4}$$

RWR_{max}: maximum relative whey release after infinite time; $t_{1/2}$: time to reach half of RWR_{max}

Syneresis of curd grains made from concentrated milk was approximated according to equation 7.5. The time constant, a, was introduced to consider the MF process applied prior to the experiments. It may be interpreted as the time required for MF to achieve a certain value of i. The higher the i-value, the higher the a-value since more time is required for MF and vice versa.

$$\frac{(t+a)}{RWR} = \frac{(t+a)}{RWR_{max}} + \frac{t_{1/2}}{RWR_{max}} \tag{7.5}$$

Furthermore, syneresis of curd grains made from concentrated and unconcentrated milk was estimated by means of equation 7.6.

$$\frac{(t+a)}{DM} = \frac{(t+a)}{DM_{max}} + \frac{t_{1/2}}{DM_{max}} \tag{7.6}$$

DM: dry matter of curd grains after time t; DM_{max}: dry matter of curd grains after infinite time

The kinetic parameters a, DM_{max}, RWR_{max} and $t_{1/2}$ were calculated via a regression procedure, using the software Sigma Plot 8.0 (SPSS Inc., Chicago, USA). For each milk system, the time to reach the equivalent dry matter found in standard milk curd grains after 45 min of syneresis $t(DM_{45min})$ was calculated by means of the estimated kinetic parameters. 45 min was chosen because this refers to a curd treatment time often applied in conventional semi-hard cheese manufacture and marks the end of the syneresis step.

7.2.7 Experimental Design

The effects of the variables i, p_1 and pH on the CF of milk gels and syneresis of rennet curd grains were studied using a central composite design according to Kleppmann (2006). The design consisted of an experimental cube considering two levels of p_1 (0 and 8 MPa), i (1 and 2) and pH (6.6 and 6.4) with a center point and an experimental star considering extreme levels. The 6th star point was not realizable because a negative homogenization pressure is not existing. Since the experiments were carried out over a period of 3 weeks the design was divided into 3 blocks considering the weekly change in raw milk composition. According to Kleppmann (2006), 1 block included the experimental star and the experimental cube was subdivided into the remainder of the blocks. The center point was weekly realized leading at least to 3 replicates of the center point. This arrangement led to 18 treatments as shown in Table 7.3.

The STATGRAPHICS Plus package (version 5.1, Statistical Graphics Corp., Rockville, USA) was used for the statistical analysis. The uncoded levels (Table 7.3) for each factor were linearly coded, i.e. the vertices of the cube from -1 to +1. From the data obtained in the experimental design, an analysis of variance (ANOVA) was done to establish the presence or absence of significant differences in CF and syneresis, considering i, p_1 and pH as factors. A second order polynomial equation was used as initial model and through step-wise regression, insignificant terms ($P > 0.05$) were eliminated.

7.3 Results

7.3.1 Effect of Milk Processing and pH on CF

The two-level factorial design was used to study the effects of i, p_1 and pH on the CF after 60 min of coagulation. The analysis of the results revealed that the effects of i, p_1 and pH were highly significant ($P < 0.01$, Table 7.1) on CF. The simplified second-order polynomial model is given in Table 7.2. R^2 for the response was highly correlated ($R^2 \geq 0.99$). In summary, CF increased when i was increased and pH decreased, whereas homogenization of the milk prior to MF decreased the gel firmness.

Table 7.1: Significance levels (P) of the analysis of variance for the effects of homogenization pressure (p_1), concentration factor of MF (i) and pH on curd firmness and syneresis of curd grains.

	i	p_1	pH	i^2	i x p_1	i x pH	p_1^2	p_1 x pH	pH^2	Blocks
F-60-value	**	**	**	NS	*	**	NS	*	*	*
RWR_{60min}	**	*	*	NS	NS	NS	*	NS	NS	NS
DM_{max}	**	*	*	**	NS	NS	NS	NS	NS	NS
$t(DM_{45min})$	**	NS	*	*	*	NS	NS	NS	NS	NS

DM_{max}: dry matter of curd grains after infinite curd treatment time (estimated according to equation 7.6); F-60-value: curd firmness after 60 min of coagulation; NS: not significant; RWR_{60min}: relative whey release of curd grains after 60 min of curd treatment time; $t(DM_{45min})$: time in min to reach the equivalent dry matter found in standard milk curd grains after 45 min of syneresis; * $P < 0.05$; ** $P < 0.01$

Table 7.2: Effects of homogenization pressure (p_1), concentration factor of MF (i) and pH on curd firmness (CF) and syneresis of curd grains; simplified second-order polynomial equations with significant coded values at P < 0.05

Equation		R^2	s.e.
F-60-value (N)	= 0.759 + 0.419 i - 0.078 p_1 + 0.118 pH + 0.023 (i x p_1) + 0.047 (i x pH) - 0.020 (p_1 x pH) - 0.011 $(pH)^2$	0.999	0.021
RWR_{60min} (%)	= 56.39 + 8.14 i - 9.70 p_1 + 2.51 pH + 6.04 $(p_1)^2$	0.847	4.45
DM_{max} (%)	= 39.89 + 7.58 i - 1.66 p_1 + 0.87 pH - 2.11 i^2	0.957	1.67
$t(DM_{45min})$	= 21.94 - 27.54 i - 4.68 pH + 4.63 $(i)^2$ - 6.26 (i x p_1)	0.946	6.89

DM_{max}: dry matter of curd grains after infinite curd treatment time; F-60-value: CF after 60 min of coagulation; RWR_{60min}: relative whey release of curd grains after 60 min of curd treatment time; R^2: determination coefficient; s.e.: standard error; $t(DM_{45min})$: time in min to reach the equivalent dry matter found in standard milk curd grains after 45 min of syneresis

7.3.2 Effect of Milk Processing and pH on Syneresis

Since milk processing and pH significantly influenced the coagulum strength, as previously shown, CT was individually determined for each milk system. CT markedly decreased when i was increased, and pH was decreased, respectively (Table 7.3). The progression of syneresis of curd grains made from different milk systems is shown in Figure 7.2.

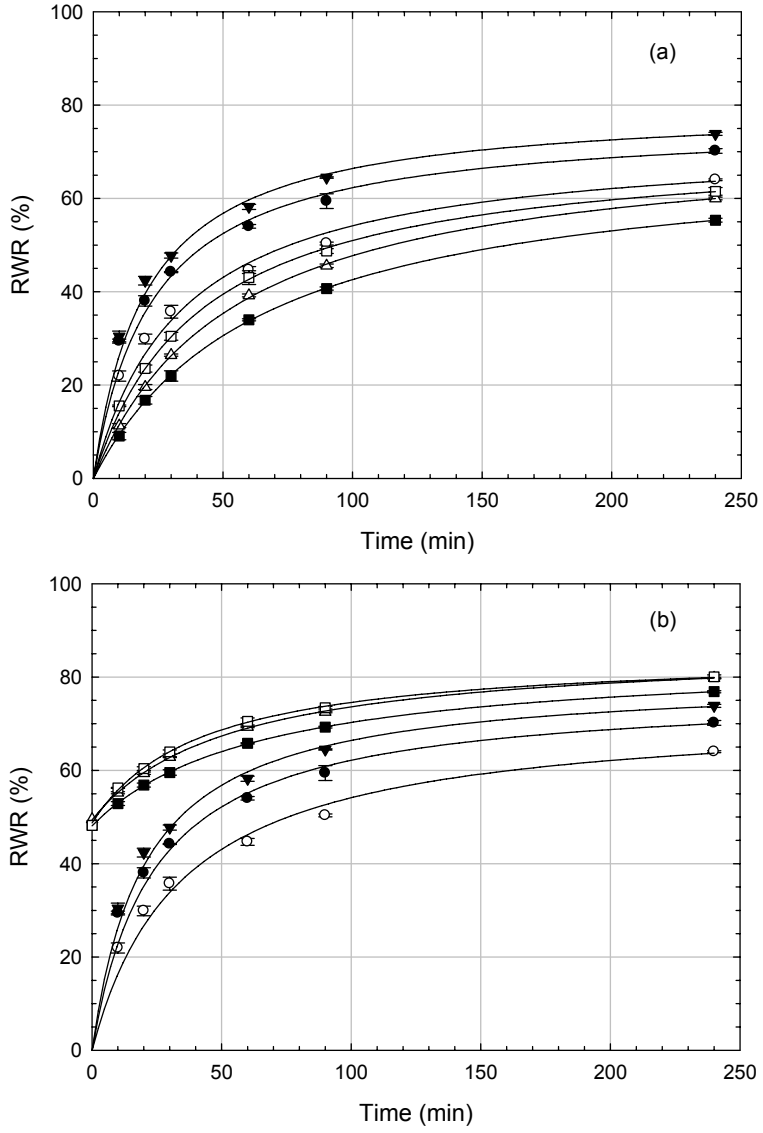

Figure 7.2: Relative whey release (RWR) of curd grains with a diameter of 11 mm at 35 °C depending on time and milk processing. Cutting of the curd was performed at comparable G' values. (a) RWR was calculated from equation 7.2 and (b) RWR was calculated according to equation 7.3. Plotted are the mean values of three measurements with standard deviation and the calculated lines according to parameters obtained from (a) equation 7.4 and (b) equation 7.5. (●, i = 1; 0 MPa; pH = 6.6; ○, i = 1; 8 MPa; pH = 6.6; ▼, i = 1; 0 MPa; pH = 6.4; △, i = 2; 0 MPa; pH = 6.6; ■, i = 2; 8 MPa; pH = 6.6; □, i = 2; 8 MPa; pH = 6.4)

In Figure 7.2a, RWR was calculated by means of equation 7.2, in which the release of permeate during MF was not considered. The curvilinear progression of syneresis is similar for the surveyed milks. Syneresis was more pronounced if pH was lowered prior to renneting. An increase in i retarded the whey release as well as an increase in p_1 (Figure 7.2a). The endpoint of syneresis (RWR_{max}), indicated by the asymptotic progression of the curve, decreased when i was increased.

The RWR values in Figure 7.2b were calculated according to equation 7.3. The values were generated from the same raw data like those presented in Figure 7.2a, but the release of permeate during MF was considered. Likewise, a decrease in pH increased syneresis and an increase in p_1 retarded the whey release of curd grains. In contrast to the presented results in Figure 7.2a, a rise in i due to MF increased the starting-point of the syneresis curves, leading to higher endpoints of syneresis (RWR_{max}) regarding the asymptotic progression of the curve. The analysis of the experimental design showed that the effect of i on RWR after 60 min of curd treatment time (RWR_{60min}) was highly significant for i and significant for p_1 and pH (Table 7.1).

The data obtained from simultaneously measuring the DM of curd grains during syneresis confirmed these findings. Figure 7.3 reveals the dependence between RWR and DM of curd grains during ongoing syneresis that can be described by the given equation. The equation was derived from two individual mathematical equations as is explained in the Appendix.

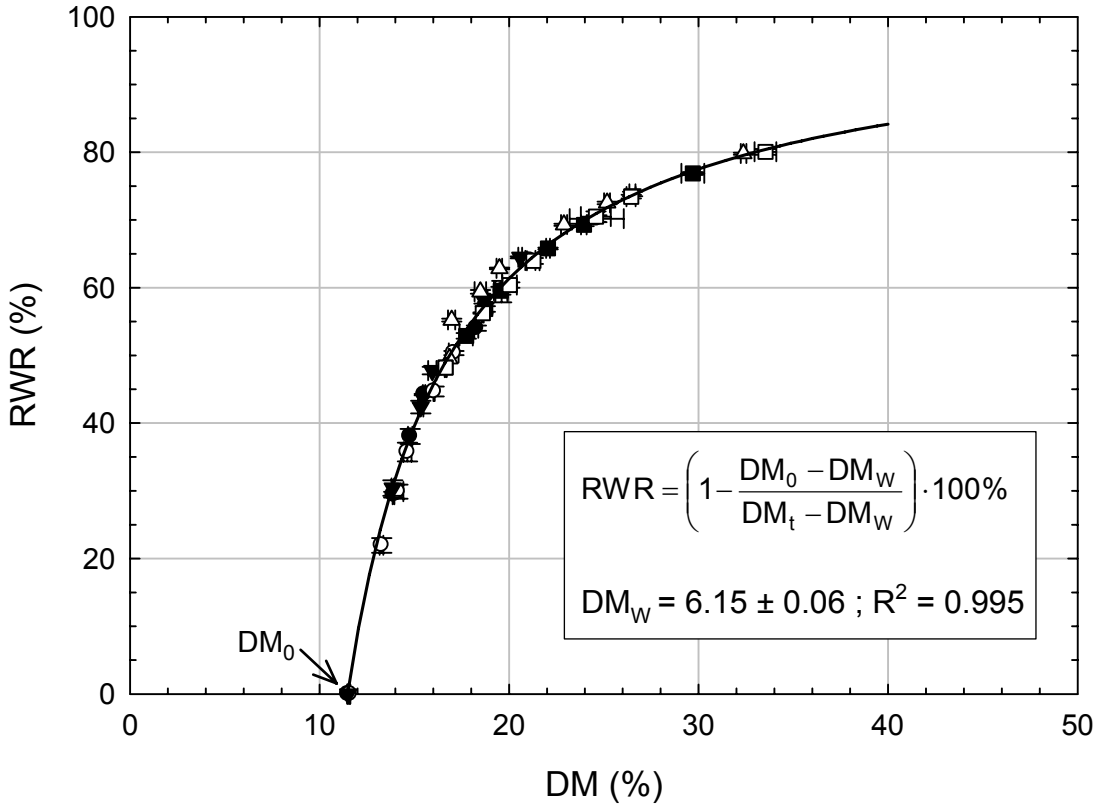

Figure 7.3: Correlation between relative whey release (RWR) of curd grains made from differently treated milk and their corresponding dry matter (DM). (●, i = 1; 0 MPa; pH = 6.6; ○, i = 1; 8 MPa; pH = 6.6; ▼, i = 1; 0 MPa; pH = 6.4; △, i = 2; 0 MPa; pH = 6.6; ■, i = 2; 8 MPa; pH = 6.6; □, i = 2; 8 MPa; pH = 6.4; DM_0: dry matter of a curd grain at the beginning of syneresis; DM_t: dry matter of a curd grain at time t; DM_W: dry matter of effluent whey)

7.3.3 Effect of Milk Processing and pH on Kinetics of Syneresis

The kinetic parameters estimated by means of equation 7.6 for curd grains with a diameter of 11 mm through a regression analysis procedure are given in Table 7.3. The proposed model fitted the experimental data well ($R^2 > 0.98$). DM_{max} characterizes the endpoint of syneresis after infinite curd treatment time and $t_{1/2}$ is the time required to reach half of DM_{max}. The analysis of the two-level factorial design showed that the effect of i and the quadratic term of i were highly significant ($P < 0.01$) on DM_{max} and significant ($P < 0.05$) for p_1 and pH (Table 7.1). The corresponding second-order polynomial model in Table 7.2 confirms the previously presented results concerning RWR. The endpoint of syneresis, DM_{max}, increased when i was increased, and pH was decreased, respectively, and decreased when p_1 was increased. R^2 for the response was satisfactory ($R^2 \geq 0.95$).

Table 7.3: Uncoded values of independent variables for the central composite design and the corresponding values with s.e. for the cutting time (CT) and kinetic parameters estimated through regression by equation 7.6.

Treatment	Block	i	p_1 (MPa)	pH	Fat (g kg^{-1})	$d_{3,2}$ ± s.d. (µm)	CT ± s.d. (min)	DM_{max} (g kg^{-1})	$t_{1/2}$ (min)	a (min)	$t(DM_{45min})$
1	1	1	0	6.4	29.5	3.370±0.013	33.3±0.3	349±10	93±10	48.9±3.9	40
2	1	2	0	6.4	58.5	n.d.	18.5±0.7	472±12	95±9	51.0±3.7	3
3	1	1	0	6.6	29.5	3.370±0.013	60.0±0.0	311±14	76±14	47.2±6.8	45
4	1	2	0	6.6	58.5	n.d.	28.9±0.2	493±22	168±23	82.4±7.1	6
5	1	1.5	0	6.5	43.0	3.414±0.005	24.4±0.4	420±9.0	113±9	55.1±3.1	22
6	1	1.5	4	6.5	41.5	0.754±0.009	20.9±0.6	369±8.0	89±8	51.9±3.3	24
7	2	0.9	4	6.5	27.3	0.768±0.027	58.8±1.9	262±8.0	64±9	49.1±5.4	70
8	2	1.5	4	6.7	43.5	0.871±0.024	48.5±0.5	378±19	155±26	93.6±9.8	33
9	2	1.5	4	6.3	43.5	0.871±0.024	16.2±0.1	398±9.0	87±8	47.2±3.2	18
10	2	1.5	4	6.5	43.5	0.871±0.024	25.0±0.4	390±7.0	103±6	57.0±2.6	22
11	2	2.5	4	6.5	73.5	0.587±0.020	23.3±0.1	455±12	101±11	77.0±5.7	-17
12	2	1.5	12	6.5	42.8	0.557±0.005	24.5±0.4	356±10	94±10	59.0±4.6	27
13	3	2	8	6.6	57.5	0.645±0.001	26.7±0.5	415±12	130±14	86.9±6.2	3
14	3	2	8	6.4	57.5	0.645±0.001	16.7±0.4	446±13	100±11	60.9±4.8	1
15	3	1	8	6.6	30.3	0.675±0.008	50.5±0.4	268±11	86±16	71.0±9.3	78
16	3	1	8	6.4	30.3	0.675±0.008	29.4±0.9	319±14	85±15	52.3±7.1	45
17	3	1.5	4	6.5	42.3	0.912±0.017	27.1±0.2	395±16	131±19	73.7±7.0	25
18	3	1.5	4	6.5	42.3	0.912±0.017	n.d.	369±9.0	97±9	59.8±4.1	23

a: time constant; DM_{max}: dry matter of curd grains after infinite curd treatment time; i: concentration factor of MF; p_1: homogenization pressure; n.d.: not determined; s.d.: standard deviation; s.e.: standard error; $t_{1/2}$: time to reach half of DM_{max}; $t(DM_{45min})$: time in min to reach the equivalent dry matter found in standard milk curd grains after 45 min of syneresis

When considering, that in conventional semi-hard cheese manufacture curd treatment often lasts for 45 min in order to reach the appropriate DM for moulding, the DM of grains made from standard milk after 45 min of curd treatment in the model system (DM_{45min} = 182.7 g kg^{-1}) can be taken as reference. By means of equation 7.6 and the kinetic parameters presented in Table 7.3, the required time t(DM_{45min}) to reach the reference value of syneresis for each milk system was calculated. The results showed that an increase of i tremendously reduced t(DM_{45min}) and the analysis of the statistical design (Table 7.1) revealed that the effect of MF was highly significant on t(DM_{45min}). t(DM_{45min}) also decreased when pH was decreased. Hence, curd treatment for highly concentrated milk, say 2.5-fold, may become obsolete (t(DM_{45min}) = -17 min, Table 7.3), since its DM prior to the syneresis experiments was DM = 200.1 g kg^{-1} compared to DM_{45min} = 182.7 g kg^{-1} of curd grains made from standard milk after 45 min of syneresis.

7.3.4 Impact of Milk Composition on RWR_{max}

An additional experiment was carried out to evaluate the influence of the milk composition on the endpoint of syneresis, RWR_{max}. For this purpose, skim milk, whole milk and homogenized whole milk was MF concentrated prior to the syneresis tests. Within each series of milk, cutting of the curd was performed at equivalent CF. Table 7.4 presents the corresponding values of RWR_{max}, calculated by means of equations 7.4 and 7.5. In summary, RWR_{max} increased when i was increased and the rise in RWR_{max} was more pronounced for curd grains made from skim milk than from whole milk. RWR_{max} was found to decrease for i less than or equal to 2.4 in the order skim milk > whole milk > homogenized whole milk.

Table 7.4: Relative whey release after infinite time (RWR_{max}) of curd grains made from different milk systems depending on the concentration factor of MF (i). pH of the milk was adjusted to pH 6.5. Within each series of milk, cutting of the curd was performed at equivalent firmness. Syneresis of the curd grains was followed at 35 °C and RWR_{max} of unconcentrated milk was estimated by equation 7.4 and for concentrated milk by equation 7.5.

Skim Milk		Whole Milk		Homogenized Whole Milk*	
i	RWR_{max}	i	RWR_{max}	i	RWR_{max}
1.0	82.2 ± 0.8	1.0	76.3 ± 1.3	1.0	73.3 ± 1.2
1.7	90.7 ± 1.0	1.7	84.6 ± 0.1	1.5	82.8 ± 0.6
2.4	94.0 ± 0.6	2.0	86.2 ± 0.3	2.1	85.4 ± 0.5
3.4	93.6 ± 0.4	2.9	86.6 ± 0.4	3.2	88.0 ± 0.7

* p_1 = 5 MPa

7.4 Discussion

7.4.1 Curd Firmness (CF)

The results obtained from the experimental design revealed that an increase in i and a decrease in pH significantly increased CF and this is consistent with results of Storry and Ford (1982), and Schreiber *et al.* (2000). Results obtained from rheometry measurement (data not shown) explain the higher F-60-values by a rise in the curd firming rate. MF increased the number of structure-forming particles and after coagulation, more bonds per time are linked within the network giving high curd firming rates and therefore high F-60-values. This is consistent with findings of Storry *et al.* (1982), Waungana *et al.* (1999) and Mishra *et al.* (2005). Furthermore, it is well known that rennet coagulation time decreases when pH is lowered (van Hooydonk *et al.* 1986). Hence, curd firming starts earlier leading to higher F-60-values. The interaction of i and pH also significantly increased CF, as expected.

Homogenization significantly decreased the F-60-value as reported by Jana *et al.* (1992) and Ghosh *et al.* (1994). Since the casein content among samples of the same concentration factor was comparable, the change in composition and structure of the milk fat globule membrane due to homogenization may partly explain this observation. Apart from the marked reduction of the average fat globule size with increasing pressures (Table 7.3), approximately 75 % of the secondary milk fat globule membrane, that supplements the native membrane after homogenization, is covered with casein (Walstra and Oortwijn 1982; Cano-Ruiz and Richter 1997). Unlike an unhomogenized fat globule, the homogenized globule is therefore incorporated into the network and if mechanical stress is applied, the casein-fat network is more flexible, lowering the resistance of the gel. Furthermore, pictures obtained by scanning electron microscopy showed that in comparison to untreated milk the protein-fat strands of microfluidized milk (very intensive homogenization) were bulky and of uneven thickness and apparently more strands ended in nodules that were not tied into the gel structure (Tosh and Dalgleish 1998).

CT was individually determined for each milk system using small amplitude oscillatory rheometry to generate comparable CF at cutting. In so doing, the significant effects of milk processing and pH on curd structure and coagulum strength are considered. Hence, syneresis should be unaffected by this variable. CT was

significantly related to the F-60-value of the individual milk sample ($r = -0.69$, $P < 0.01$), demonstrating that a high F-60-value results in a reduction of CT and vice versa.

7.4.2 Syneresis

Syneresis of curd grains is significantly influenced by pH as reported by several workers (Patel *et al.* 1972; Pearse and Mackinlay 1989; Grundelius *et al.* 2000). Homogenization retarded the whey release and this is in agreement with findings of Humbert *et al.* (1980), Green *et al.* (1983) and Ghosh *et al.* (1994). Walstra *et al.* (1985) attributed this effect to the alteration in the protein-fat structure of the curd from homogenized milk. The fat globules are incorporated into the network due to their secondary milk fat globule membrane, thus hindering the shrinkage of the network.

Syneresis of curd grains made from concentrated milk due to MF or UF is often described without consideration of the permeate release during the filtration process. Some authors (Peri *et al.* 1985; Pearse and Mackinlay 1989; Caron *et al.* 2001) reported a decrease in whey release when the casein content was increased. This is in agreement with the results presented in Figure 7.2a. Both, syneresis rate and the endpoint of syneresis were reduced when i was increased. Van Dijk and Walstra (1986) explained this observation by a decrease in the permeability of the network. However, consideration of the permeate release during MF in the calculation of syneresis delivered an opposite result concerning the endpoint of syneresis (Figure 7.2b). The maximum whey release increased when the concentration factor was increased and this was confirmed by the additional experiment conducted on skim milk, whole milk and homogenized whole milk (Table 7.4). Curd grains made from skim milk had the highest RWR_{max} value and this is explainable by two effects: the higher water content of the coagulated milk and the existence of a network consisting only of casein strands. This results in the formation of a denser network with consequently greater shrinkage effects as likewise reported by Casiraghi *et al.* (1987). The greater shrinkage may be explained by the absence of fat globules within the network, usually acting as spacers between the casein strands.

If MF is implemented in the cheesemaking procedure, the calculation of syneresis by means of equation 7.3 is preferable to monitor the whey release since the RWR

values of the curd grains made from differently treated milk are yet comparable (Figure 7.2b). Likewise, the determination of the DM of curd grains offers another promising method to describe syneresis considering permeate release during MF. The obtained dependence between RWR and the grains' DM (Figure 7.3) confirmed the fact, that syneresis is a mild dehydration process, since the curve progression is typical for a drying process. Furthermore, the proposed equation considers the fact that unlike water, the expelled whey contained a certain amount of DM. Since the DM content depends on syneresis time (Castillo *et al.* 2000) and porosity of the curd grain, it may be possible to correlate the calculated DM of the whey at a certain treatment time with factors that influence syneresis.

The proposed kinetic models (equations 7.4 to 7.6) were satisfactorily fitted to the experimental data ($R^2 > 0.98$) as previously reported by Thomann *et al.* (2006). The impact of i, p_1 and pH on syneresis could be characterized by means of the kinetic parameters, especially in studying the endpoint of syneresis, DM_{max} and RWR_{max}, respectively.

7.4.3 Combination of the Variables for Process Adaptation

From the simplified equations in Table 7.2, lines of equal effects were calculated.

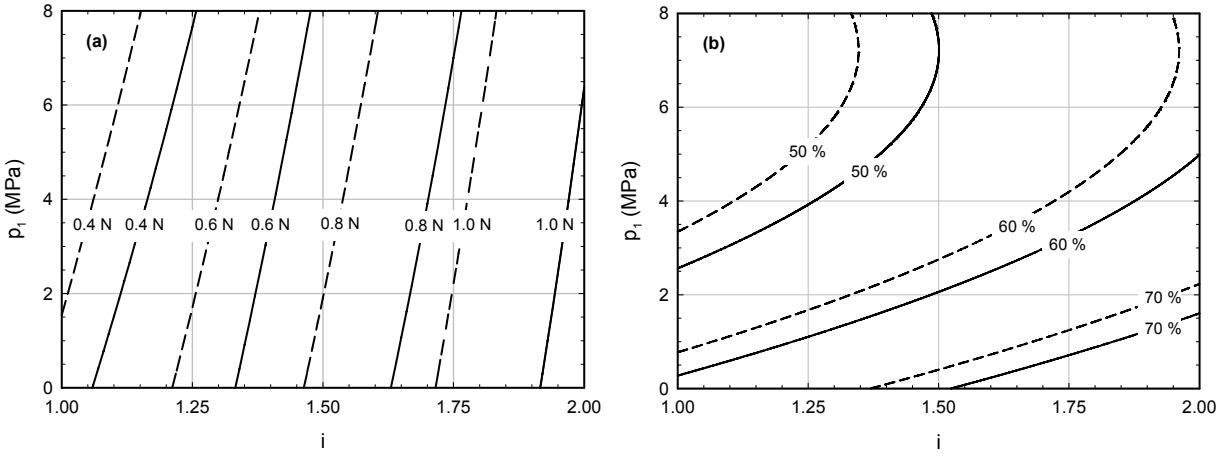

Figure 7.4: Lines of (a) equal curd firmness (F-60-value) as a function of concentration factor of MF (i) and p_1 and (b) equal syneresis of curd grains with a diameter of 11 mm at 35 °C after 60 min of curd treatment (RWR_{60min}) as a function of i and pH. (—: pH 6.6; ----: pH 6.5)

By means of these specific lines, which are valid only within the set experimental conditions, it is demonstrated that MF and p_1 can be combined to reach certain CF and syneresis values. When considering that 0.4 N refers to a CF of the standard,

homogenization may be carried out at e.g. 8 MPa prior to concentrating 1.25-fold, respectively 1.15-fold if pH was lowered to 6.5 (Figure 7.4a). Syneresis of 50 % after 60 min of curd treatment is reached if milk is homogenized at e.g. 4 MPa or 5 MPa if pH is decreased to 6.5, prior to concentrating 1.25-fold (Figure 7.4b).

7.5 Conclusion

The main objective of this study was to investigate the impact of homogenization, MF, pH and their interaction on syneresis of curd grains made from rennet-induced milk gels. For generating comparable conditions at the start of the experiments, CF was characterized and CT was individually determined for each milk system.

(i) Results obtained from texture analysis indicate that weak CF due to homogenization can be compensated if MF is additionally applied.

(ii) A model was proposed that considered the permeate release during MF in the calculation of syneresis. In addition, syneresis was successfully described by measuring the DM of the curd grains depending on curd treatment time. Both methods allow a comparison between syneresis values obtained from curd grains made from concentrated and unconcentrated milks.

(iii) The proposed kinetic models were satisfactorily fitted to the experimental data ($R^2 > 0.98$). The endpoint of syneresis, DM_{max} and RWR_{max}, varied with i and milk composition. Curd grains made from skim milk had the highest RWR_{max} value. It is to be assumed, that differences in curd microstructure affect syneresis since cutting was performed at equal CF.

(iv) If considering the milk volume prior to MF, the amount of cheesemaking additives decreased with increasing concentration of the milk. Although less rennet was given to the concentrated milk, CT tremendously decreased with increasing i. It was shown that with increasing milk concentration and/or decreasing pH values less curd treatment time was needed to reach the equivalent dry matter found in standard milk curd grains after 45 min of syneresis t(DM_{45min}). Hence, costs for cheesemaking additives and processing

time can be saved by integrating MF technology into the manufacture procedure.

The combined study highlights the influence of milk processing and pH on CF and syneresis and provides useful information concerning the implementation and combination of innovative technologies in conventional semi-hard cheese manufacture. Reaching CF and syneresis comparable to untreated milk used in conventional cheesemaking meets one claim of the industry when implementing both technologies in the manufacture process, since consistency and quality of the ripened cheese are expected to be unchanged.

7.6 Acknowledgment

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7.8 Appendix

The dependence of the relative whey release (RWR) and dry matter was derived from equation 7.2 and from equation 7.7 that presents a mass balance considering the weight, m_0, and the dry matter, DM_0, of a curd grain at the beginning of syneresis. The weight of the curd grain at time t, m_t, and the corresponding absolute dry matter decreases during syneresis, since mass (m_0 - m_t) and dry matter (DM_W) are lost with the effluent whey.

$$m_0 \cdot DM_0 = m_t \cdot DM_t + (m_0 - m_t) \cdot DM_W \tag{7.7}$$

Equation 7.7 is transformed to equation 7.8

$$1 - \frac{m_t}{m_0} = 1 - \frac{DM_0 - DM_W}{DM_t - DM_W} \tag{7.8}$$

and from the insertion of equation 7.2 in equation 7.8, equation 7.9 is obtained.

$$RWR = \left(1 - \frac{DM_0 - DM_W}{DM_t - DM_W}\right) \cdot 100\% \tag{7.9}$$

8 FEASIBILITY STUDY FOR SEMI-HARD CHEESE MANUFACTURE: YIELD AND FUNCTIONALITY OF FULL-FAT SEMI-HARD CHEESE AS INFLUENCED BY THE COMBINATION OF MICROFILTRATION AND HOMOGENIZATION

Abstract

Two series of full-fat semi-hard cheese, A and B, were made in which the milk fat and protein level was increased from 3.0 to approximately 6.0 % by means of microfiltration (MF). From the results gained in series A, the cheese manufacture procedure in series B was modified. The objective of the study was to determine the effects of MF, homogenization (0 and 8 MPa) prior to MF and cheese manufacture procedure on cheese yield and functionality. Actual cheese yield markedly increased by 3 to 14 % compared to the standard (cheese made from pasteurized, untreated milk) within both series with increasing protein and fat contents of the cheese milk. This effect was even intensified if the milk was homogenized prior to MF, since fat and protein recovery increased. Dry matter content of manufactured cheeses following the conventional cheesemaking procedure (series A) increased compared to the standard. The cheeses appear harder and did not fracture. Therefore, modifications in the cheesemaking procedure (series B) were made to overcome these problems. Based on preliminary studies, curd treatment time was individually calculated for each milk system to generate a comparable degree of syneresis at the end of curd treatment. Following the modified procedure, compositions of curd, raw cheese and ripened cheese among the individual trials became comparable. In addition, homogenization clearly altered cheese colour, and decreased cheese meltability, browning during heating and oiling off.

Keywords: *Curd Treatment; Homogenization; Meltability; Microfiltration; Semi-Hard Cheese; Texture*

8.1 Introduction

The most important factor affecting cheese yield is the milk composition, in particular the concentrations of fat and protein, which together constitute ~92 % of the dry matter (DM) of semi-hard cheese. Yield and manufacturing efficiency (e.g., percentage recovery of milk fat or protein to cheese) are major determinants of the profitability accruing to cheese manufacturing plants (Guinee *et al.* 2006). Microfiltration (MF), a new membrane technique, may increase the throughput of existing cheese manufacturing facilities up to 80 % (Thomet *et al.* 2004). Unlike ultrafiltration (UF), textural and flavour defects in cheeses are not expected since the use of MF retentate leads to composition very similar to that of conventional cheese curd (Papadatos *et al.* 2003). The MF approach is not proposed to increase cheese yield efficiency (i.e., more cheese from the same amount of unconcentrated milk), because enhanced retention of serum proteins in the cheese matrix is not expected (Neocleous *et al.* 2002a).

Homogenization of milk and cream has been successfully applied in the manufacture of several cheese types, especially in soft cheese manufacture. Metzger and Mistry (1994) and Nair *et al.* (2000) reported a significant increase in Cheddar cheese yield with homogenization of cream prior to the experiments. This was attributed to enhanced fat and protein recovery due to the secondary fat globule membrane, interacting with casein during rennet gel formation.

Besides the promising effects of homogenization on component recovery and cheese yield, respectively, and of MF on profitability considering the increase in throughput, inconsistent results concerning cheese functionality are reported. MF is supposed to increase cheese hardness and to decrease cheese flavour due to reduced proteolysis (Neocleous *et al.* 2002b) while homogenization seems to cause either flavour defects in the ripened cheese (Jana and Upadhyay 1992), or to influence cheese meltability (Tunick *et al.* 1993; Nair *et al.* 2000) or colour (Lemay *et al.* 1994; Rudan *et al.* 1998).

Very limited research has been established on the combination of homogenization and concentration of milk prior to cheesemaking. One study by Green *et al.* (1983) revealed that the composition of Cheddar cheese made from homogenized milk prior to UF was improved, because of increased fat and moisture retention. Previous

results in Chapter 6 and 7 illuminated the interrelated effects of homogenization and MF on rennet gel formation, gel texture and syneresis of curd grains during curd treatment. From these results it can be drawn, that conventional cheesemaking procedure can be adapted and simplified. So, the objective was to study in pilot experiments:

(i) The effect of conventional and modified cheesemaking procedure on cheese yield and functionality.
(ii) The influence of homogenization and MF of milk prior to cheesemaking on cheese composition, yield and functionality.

8.2 Materials and Methods

8.2.1 Materials

Raw Milk

For all experiments, whole raw bovine milk was obtained from the Research Station Meiereihof (University of Hohenheim, Germany).

Material Used for the Cheesemaking Trials

- Calcium chloride solution: Calcium chloride (1.02382.1000, VWR, Darmstadt, Germany) diluted with distilled water in a ratio 1:4
- Starter culture solution: Prior to the cheesemaking trial, 100 g of frozen direct vat starter culture (Probat 322 FRO 500 DCU, Danisco, Niebüll, Germany) were thawed in 300 g of unconcentrated milk and stored at 4 °C until further use.
- Lactic acid solution: lactic acid (1.00366.2500, VWR, Darmstadt, Germany) diluted with distilled water to a concentration of 9 %
- Latex coating (IP Ingredients GmbH, Süderlügum, Germany)
- Lysozyme (Afilact Fluid, Chr. Hansen, Hoersholm, Denmark)
- Rennet solution: rennet (ChyMax Plus, 190 IMCU/mL, Chr. Hansen, Hoersholm, Denmark) diluted with distilled water in a ratio 1:19
- Ripening foil (BK4L, Cryovac, Norderstedt, Germany)

8.2.2 Methods

Two series of experiments, A and B, were undertaken to study the effects of increasing milk fat and protein level (from 3.3 to 6.2 %), homogenization pressure (0 and 8 MPa) and cheese processing on cheese yield and functionality. In series A, cheese was manufactured following the conventional procedure. From the experiences gained in series A, the procedure was modified and simplified in series B. Cheesemaking trials in each experimental series were performed within three incoherent weeks.

Processing of Milks for Cheese Manufacture

The ratio of fat to protein in the milk was adjusted to ~0.9 by adding skim milk to the raw milk. The standardized raw milk was immediately processed after pasteurization to produce four types of milk for experimental purposes (Figure 8.2): untreated milk, homogenized and unconcentrated milk, unhomogenized MF concentrated milk and homogenized MF concentrated milk. The mean composition of the standardized, pasteurized cheese milk (refered to as standard), used in series A and B for homogenization, microfiltration and further cheesemaking, presents Table 8.1.

Table 8.1: Means of compositions of standardized, pasteurized cheese milk (standard) used in semi-hard cheese manufacture[1].

Experimental series	Dry Matter (%)	Protein (%)	Fat (%)	Calcium (%)
A	$(11.49 \pm 0.12)^a$	$(3.34 \pm 0.08)^a$	$(2.96 \pm 0.05)^a$	$(0.117 \pm 0.004)^a$
B	$(11.20 \pm 0.20)^b$	$(3.28 \pm 0.05)^a$	$(2.96 \pm 0.06)^a$	$(0.118 \pm 0.002)^a$

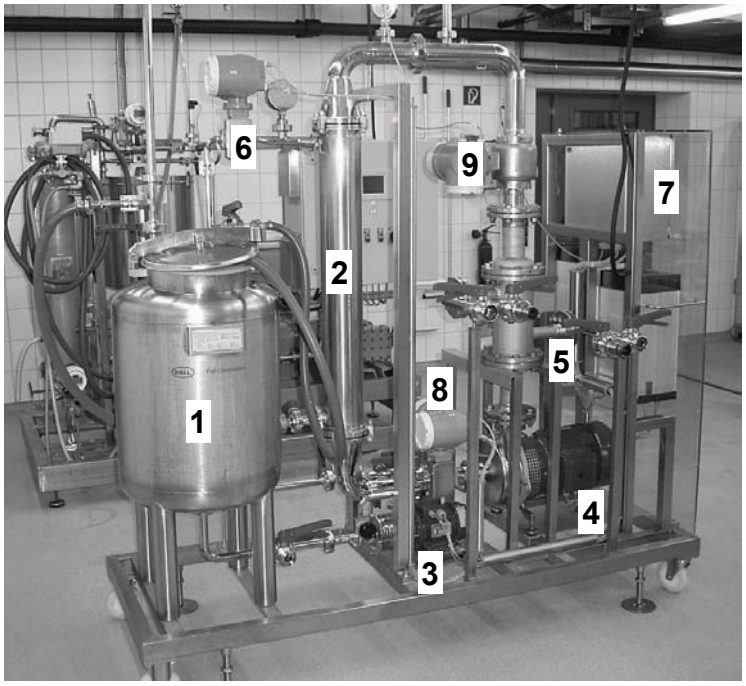
[a, b]values in a column with a common superscript letter do not differ significantly ($P > 0.05$); [1]presented values are means of three independent cheesemaking experiments

Pasteurization (74 °C for 22 s) and homogenization were carried out prior to MF in a pilot-scale heating plant (Asepto, Dinkelscherben, Germany). Homogenization was carried out at 65 °C applying a pressure (p_1) of 8 MPa using an orifice valve provided by the University of Karlsruhe. In doing so, the volume-to-surface mean diameter was reduced to about 0.7 µm. Back pressure (p_2) was realized using a needle valve. The ratio of p_2 to p_1 (Thoma Number) was adjusted to 0.15. MF concentration up to 2-fold was carried out using a pilot-scale membrane processing unit (model TFF, Pall SeitzSchenk, Waldstetten, Germany) equipped with a ceramic Membralox gradient of permeability (GP) membrane (Type 7P-1940GP, Pall Exekia, Bazet, France) having

an average pore size distribution of 0.1 µm and a total membrane area of 1.69 m^2 (Figure 8.1). MF was carried out at 52 ± 1 °C applying a transmembrane pressure of 10^5 Pa. The pressure drop along the membrane was adjusted to $2 \cdot 10^5$ Pa giving a wall shear stress of about 199 Pa. The concentration factor of MF (i) was calculated by the fat ratio of the retentate to standard milk.

$$i = \frac{Fat_{Retentate}\ (\%)}{Fat_{Standard}\ (\%)} \tag{8.1}$$

MF was carried out either on the same day or on the following day. After the individual treatment, milk and retentate were immediately cooled down and stored in a cooling chamber at 6 - 8 °C until cheesemaking on day two or three.



1: Feed Tank
2: MF Membralox GP Membrane
3: Feed Pump
4: Circulation Pump
5: Heat Exchanger
6: Volume Flow Permeate
7: Registration
8: Volume Flow Feed
9: Volume Flow Loop

Figure 8.1: Pilot-scale membrane processing unit at the Dairy for Research and Training, University of Hohenheim.

Parameters monitored during Cheesemaking

During cheesemaking, pH, temperature and time was recorded. The pH of milk and whey was measured with an electrode (pH 522, SCHOTT, Mainz, Germany) that was calibrated at pH 6.96 and pH 4.01 at 35 °C. All samples were tempered at 30 to 35 °C at time of measurement.

Sampling and Sample Preparation

Milk and MF retentate were sampled prior to cheesemaking. All whey from each batch was collected from the beginning of curd treatment up to the end of pressing and was finally weighed. Chemical analyses of the whey were carried out after allowing the thoroughly mixed whey to rest for a short while to avoid carry over of curd fines.

At the end of the curd treatment step, approximately 50 g of curd was removed from the batch for chemical analysis. The curd was transferred to a plastic box, stored at 4 to 6 °C and since whey was expelled during storage due to ongoing syneresis, the curd and whey was thoroughly homogenized directly before analysis with an Ultraturrax (Polytron System PT 2100, Kinematica AG, Littau-Luzern, Switzerland).

Raw cheese was sampled (50 to 100 g) before brining at day one after manufacture. The outer, dryer parts of the sample were removed. The sample was afterwards grated and if analysis was not immediately carried out, stored at 4 to 6 °C in closable sample vials. The same procedure was performed for ripened cheese after 4 and 8 weeks of storage.

Chemical Analyses

Milk, retentate and whey composition. The dry matter was determined at 90 °C using an infrared dryer (Moisture Analyzer MA30, Sartorius, Göttingen, Germany). Based on the Dumas method DIN 10467, nitrogen was determined using a Leco FP-528 (Leco Instrumente GmbH, Mönchengladbach, Germany). Total protein was calculated by multiplying the nitrogen content with the milk specific factor of 6.38. The fat content was measured by the Gerber standard method (VDLUFA, C 15.3.2, 2003). The total calcium content was analyzed according to method C10.6.8 (VDLUFA 2003). All analyses were at least performed in duplicate.

Curd, raw cheese and ripened cheese composition. Moisture content was determined gravimetrically by drying approximately 3 g of sample at 102 °C in a drying oven for at least 16 hours (C35.3, VDLUFA 2003). Fat, protein and calcium content were determined by the van Gulik method (C15.3.8, VDLUFA 2003), the Dumas method DIN 10467 and the atomic absorption spectroscopy (Pollmann 1991), respectively. Proteolysis was determined according to Kuchroo and Fox (1982) and

calculated from the ratio of the water soluble protein content to total protein content of the cheese. Fat was determined in duplicate, while triplicate analysis were done for all other components.

Fat, Protein and Dry Matter Recovery

A mass balance was conducted on each batch of cheese. The weights of all inputs (milk, retentate, water, culture and $CaCl_2$-solution, lactic acid) and outputs (curd, raw cheese, whey) were determined to the nearest g during the experiment. The actual percentage fat recovery in the raw cheese was calculated, as described Neocleous *et al.* (2002a), as the weight of fat present in the raw cheese, divided by the fat present in the original cheese milk and retentate, respectively. In order to compare the recovery in the raw cheese made either from concentrated or unconcentrated milk, calculation was performed according to equation 8.2. This value was called "adjusted recovery" (ad Rc).

$$\text{ad Rc} = \frac{m_{\text{Raw Cheese}} \times \%\,\text{Fat}_{\text{Raw Cheese}}}{m_{\text{Re tentate}} \times i \times \%\,\text{Fat}_{\text{unconcentrated Milk}}} \times 100\% \qquad (8.2)$$

$m_{Retentate}$: weight of retentate in kg used for cheese manufacture; i: concentration factor of MF

In analogy, the protein and dry matter recovery was calculated.

Yield of raw cheese

Actual cheese yield, Y_a, was estimated for each batch of cheese as weight of raw cheese (plus curd samples taken during the cheese making process) divided by the weight of original cheese milk (minus the weight of the milk samples removed from the vat before rennet addition). In the case of concentrated milk, the adjusted actual yield, Y_a*, was calculated from:

$$Y_a^* = \frac{m_{\text{Raw Cheese}} + m_{\text{Curd Sample}}}{m_{\text{Re tentate}} \times i} \times 100\% \qquad (8.3)$$

Three other calculations for cheese yield were applied according to Guinee *et al.* (2006). (i) Y_{afpam}, actual yield per 100 kg of cheese milk normalized to reference levels of fat (2.96 %) and protein (3.3 %) at the protein to fat ratio of the standardized milks (□0.90); Y_{afpam} eliminates the effects of differences in milk composition and,

hence, allows to compare yields. This yield, referred to as actual yield (Y_a) per 100 kg of fat (f) and protein (p) adjusted milk (am), was determined by using equation 8.4:

$$Y_{afpam} = Y_a \times \frac{F_{rm} + P_{rm}}{F_{cm} + P_{cm}} \tag{8.4}$$

where F_{cm} and P_{cm} correspond to the actual fat and protein contents of the cheese milk, and F_{rm} and P_{rm} to the percentages fat and protein in the standard cheese milk (i.e., 2.96 and 3.34 %), respectively. (ii) Y_{ma}, moisture-adjusted cheese yield (kg/ 100 kg of cheese milk); Y_{ma} eliminates the effect of differences in cheese moisture to yield and, hence, allows to compare yield of differently manufactured cheeses.

$$Y_{ma} = Y_a \times \left(\frac{100 - M_a}{100 - M_r} \right) \tag{8.5}$$

M_a and M_r correspond to the actual moisture and reference moisture (49.54 and 52.23 %), respectively. (iii) Y_{mafpam} represents the moisture-adjusted cheese yield (Y_{ma}) per 100 kg of cheese milk related to the reference level of fat (2.96 %) and protein (3.34 %) (kg/100 kg of normalized cheese milk). The use of this equation (eq. 8.6) allows to determine the direct effect of treatment on cheese yield, without interfering effects of differences in milk composition and cheese moisture.

$$Y_{mafpam} = Y_{afpam} \times \left(\frac{100 - M_a}{100 - M_r} \right) \tag{8.6}$$

Texture Properties

Milk Preparation. For the rheological assays in series A, 0.1 % (v/w) of calcium chloride solution was added to milk and retentate, respectively. The sample was warmed up to 30 °C and pH was adjusted with lactic acid to pH 6.6. In series B, the standard milk (untreated, pasteurized milk) was prepared as described afore. No calcium chloride solution was added to the retentates in series B and pH adjustment to 6.6 for the retentates was undertaken at 35 °C. Each sample was afterwards equilibrated at the appropriate temperature for 30 min in a water bath. For coagulation, 0.44 % (v/w) of rennet solution was added to the sample.

Rheometry. A Bohlin CS10 controlled-stress rheometer (Bohlin Instruments, Pforzheim, Germany) equipped with a double-gap device (DG40/50, Bohlin

Instruments, Pforzheim, Germany) was used for studying the viscoelastic properties of the rennet-induced milk gels. After addition of rennet, 30 mL of the sample was transferred to the rheometer geometry and gel formation was monitored by measuring storage modulus (G'), loss modulus (G'') and related phase angle δ = arc tan (G''/G') at 1 Hz with a strain amplitude of γ = 0.01. A solvent trap was used to avoid water loss and incrustation. The G' value of the standard milk in series A and B, and likewise for all retentates in series A, was measured as a function of time at 30 °C. In series B, rheological measurements of the retentates were carried out at 35 °C. The G' value of the standard milk measured after 60 min of coagulation was taken as reference in order to adapt the cutting time (CT) of the corresponding milk samples. In so doing, cutting of the gel in the cheese vat was initiated at comparable gel strengths. All experiments were performed at least in duplicate.

Texture analyses of the gel. The analysis was performed according to Schreiber and Hinrichs (2000). 60 g of milk, portioned in a 100 mL glass beaker, was used and after 60 min of coagulation, force-distance curves were measured using a texture analyzer (Z2.5/TS1S, Zwick, Ulm, Germany) equipped with a load cell of 20 N. Standard milk and retentates in series A and standard milk in series B were coagulated at 30 °C, whereas retentates in series B were coagulated at 35 °C. Each point was determined fivefold.

Cheese Preparation. Cylindrical cheese samples with a diameter of 11 mm were cut at ripening temperature (13 - 14 °C) with a borer fixed in a milling machine. The cylinders were cut to a defined length of 15 mm using two parallel wires. The samples were weighed, packed in cling film and stored at 8 to 10 °C in a fridge until measuring on the next day. At least, four samples per cheese were obtained.

Texture analyses of the cheese. The texture profile was determined using a test up to 66 % compression (Eberhard 1985). Force-distance curves were generated at room temperature using a texture analyzer (Z2.5/TS1S, Zwick, Ulm, Germany) equipped with a load cell of 20 N or 1 kN. A cylindrical geometry with a diameter of 24 mm compressed the sample with a velocity of 50 mm/min to a height of 5 mm. From these curves fracturability (F_B), representing the peak force at which the sample fractures, deformation at fracture (D_B) and the force at 33 % deformation (F_D) were determined. The cheeses were evaluated after four and eight weeks of ripening.

Cheese Colour and Meltability

The colour of the unmelted and melted cheese sample was measured at five different places of the sample using a chromameter (CR-300, Minolta, Carrieres-Sur-Seine, France). The yellow-index, Y_i, was calculated by means of the determined L*a*b values (Rohm and Jaros 1996).

Meltability of cheese was determined by the covered Schreiber test (Altan *et al.* 2005). A cylinder with a diameter of 36 mm was cut with a cheese borer from the centre of the cheese and cheese discs of ~5 mm height, corresponding to a weight of ~5.5 g, were obtained. Each disc was placed in the centre of a glass Petri dish, which was covered and heated in an oven for 8 min at 232 °C. After cooling, the expansion of the cheese samples was measured.

8.3 Results and Discussion

8.3.1 Series A

8.3.1.1 Milk Composition

The standard milk was processed as previously described to obtain homogenized and unhomogenized milks of different protein and fat levels due to microfiltration. The mean composition of these milks is given in Table 8.2.

Table 8.2: Composition of processed milks used in semi-hard cheese manufacture. The presented values are means of at least duplicate measurements.

	Standard[1] n.h., i=1	n.h., i=1.98	8MPa, i=1	8MPa, i=1.24	8MPa, i=1.82	8MPa, i=1.93
Dry Matter (%)	11.49±0.12	16.82±0.03	11.61±0.02	12.93±0.09	16.62±0.04	16.65±0.05
Protein (%)	3.34±0.08	5.92	3.26	4.07±0.03	5.90±0.05	5.78
Fat (%)	2.96±0.05	5.85	3.03	3.60	5.40	5.75
Calcium (%)	0.117±0.004	0.175±0.008	0.114±0.001	0.148±0.006	0.217±0.01	0.198±0.005

[1]presented values are means of three independent cheesemaking experiments; i: concentration factor of MF; n.h.: not homogenized

With increasing concentration factor, contents of dry matter, protein and fat increased. Since more than 60 % of the milk's calcium is associated with the casein

micelle (Walstra and Jenness 1984; Zoon *et al.* 1988c), the increase of protein and especially of casein due to MF is correlated with an increasing calcium content.

8.3.1.2 Semi-Hard Cheese Manufacture

On the first or second day after milk processing two to three batches of full-fat semi-hard cheese were manufactured. For each batch, at least 7 kg of treated cheese milk was used. The cheesemaking procedure based on parameters applied in conventional cheesemaking is shown in Figure 8.2.

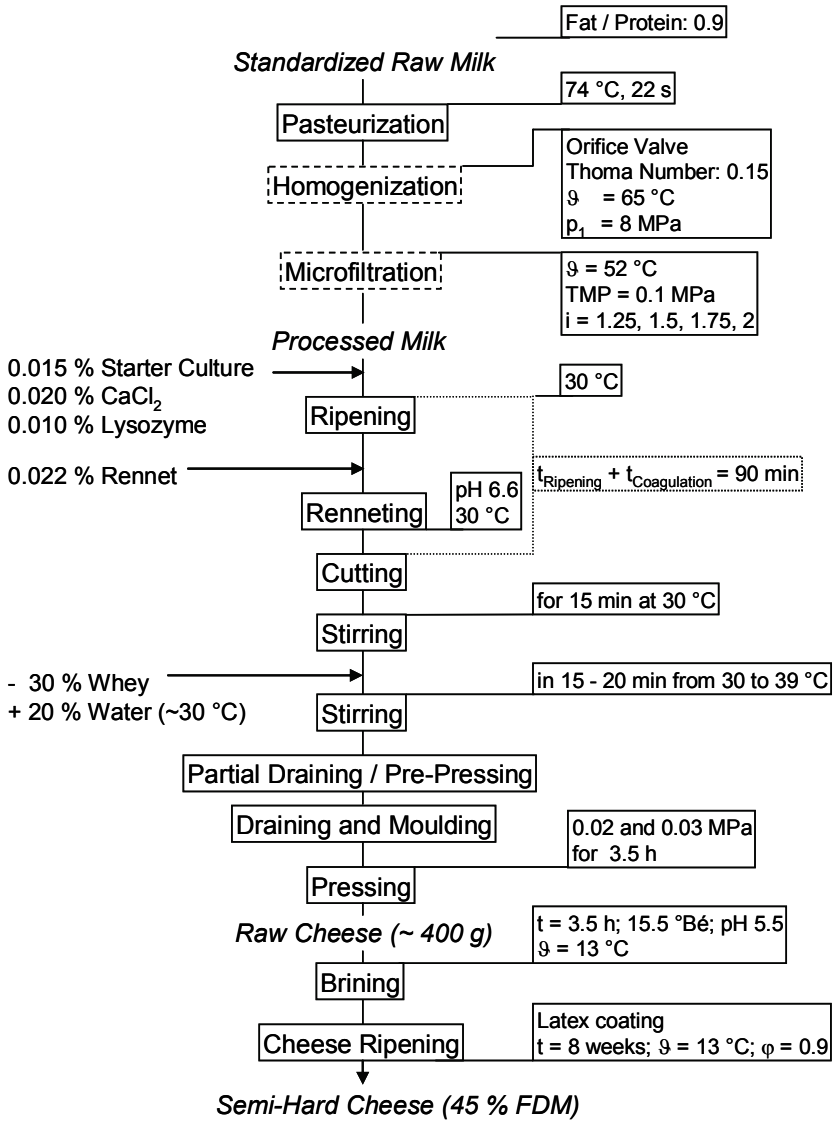


Figure 8.2: Flow sheet for processing of raw standardized milk and semi-hard cheese manufacture. (: optional; FDM: fat-in-dry-matter; i: concentration factor of MF; p_1: homogenization pressure; t: time; TMP: transmembrane pressure)

After gently warming up the milk to 30 °C, the prepared starter culture was added in a concentration of 0.015 % (w/w). Calcium chloride solution and lysozyme were

simultaneously given to the cheese milk in a concentration of 0.1 % (v/w) and 0.01 % (v/w), respectively. The milk was left to pre-ripen for 30 min and was adjusted, if necessary, with lactic acid to pH 6.6 prior to renneting. After the addition of 0.44 % (v/w) of rennet solution the standard milk was coagulated for 60 min. For retentates, the individual coagulation time was determined in the rheometer as previously described in order to cut the gels at comparable gel strengths. To allow similar pre-ripening conditions and thus activity of starter bacteria, the pre-ripening phase of the individual milk was prolonged, so that the sum of ripening and coagulation time remained constant (90 min).

The coagulated milk was cut with a wired cutting device into curd grains with an average size of 5 to 10 mm. The curd/whey mixture was gently stirred for 15 min at 30 °C, followed by drainage of 30 % of whey and addition of 20 % of tempered water (30 °C). Again, the curd/whey mixture was stirred for 15 to 20 min, while the temperature was gradually increased from 30 to 39 °C. After 5 min of curd healing and partial drainage of whey, the curd was transferred into a pre-pressing device and pressed under whey for 20 min applying a pressure of about 1 kPa. The fused curd was weighed and cut into equivalent parts. Pressing, brining and ripening were performed as is depicted in Figure 8.2. The cheeses were coated with latex three days after manufacture.

Following this procedure, the basic parameters applied in the individual cheesemaking trials as affected by milk treatment are presented in Table 8.3. The amount of added calcium, culture and rennet decreased with increasing concentration of the milk, since calculation of the ratios was based on the milk volume prior to MF and not on the milk volume taken for cheesemaking. Coagulation of all milks was initiated at 30 °C and in agreement with previous results (Chapter 6) and other authors (Zoon *et al.* 1988a; Guinee *et al.* 1994; Guinee *et al.* 2006), increasing the milk protein level enhanced particularly the curd firming rate (the change of G' with time), allowing earlier cutting of the milk gel. For example, 1.98-fold MF-concentrated milk was cut after 29 min compared to 60 min for the standard (both not homogenized).

The difference between CT of 2-fold concentrated retentates from homogenized and unhomogenized milk was neglectable. Since the ripening time was prolonged in the

case of a shortened coagulation time and curd treatment time was held constant, the starter-to-drain-time (S-to-D) for all trials was similar.

Table 8.3: Effect of homogenization and microfiltration on cheesemaking parameters.

	Standard n.h., i=1	n.h., i=1.98	8MPa, i=1	8MPa, i=1.24	8MPa, i=1.82	8MPa, i=1.93
Calcium (%)[f]	0.020	0.010	0.020	0.016	0.011	0.010
Rennet (%)[f]	0.022	0.011	0.022	0.018	0.012	0.011
Culture (%)[f]	0.015	0.008	0.015	0.012	0.008	0.008
$\vartheta_{Process}$ (°C)	30-39	30-39	30-39	30-39	30-39	30-39
RT (min)	30	61	30	50	62	60
CT (min)[*]	60	29	60	40	28	30
CTT (min)	45	45	45	45	45	45
S-to-D (min)	135	135	135	135	135	135
Whey (%)[**]	-30	-30	-30	-30	-30	-30
Water (%)	20	20	20	20	20	20

CTT: curd treatment time; i: concentration factor of MF; n.h.: not homogenized; RT: ripening time; S-to-D: starter-to-drain time; ϑ: temperature; [f]calculation of the concentration was performed by considering the original volume of milk before MF; [*]cutting time (CT) after rennet addition was determined using low-amplitude strain oscillation rheometry; [**]drained whey before water addition

8.3.1.3 Composition of Curd, Raw Cheese and Whey

The compositions of curd, raw cheese and whey are presented in Table 8.4. The values of the standard are means of three independent cheesemaking experiments and considering the low standard deviations, reproducibility of cheesemaking was given.

The dry matter content of curd made from highly concentrated milks (i > 1.8) increased compared to the standard, since curd treatment time was held constant. This is consistent with results previously shown in Chapter 7. The higher the dry matter of the retentate (Table 8.2) and of the curd grains at the beginning of syneresis, the higher the dry matter of the grains at the end of syneresis. This effect was independent whether the milk was homogenized prior to MF or not. The raw cheese composition indicate that this effect was conserved, since the corresponding dry matter, fat and protein content increased compared to the standard.

Dry matter, fat and protein losses in the effluent whey markedly increased above i = 1.24 with increasing protein and fat level as reported by Guinee *et al.* (1994). This effect was particularly pronounced if retentate of unhomogenized milk was used for

cheesemaking. Reduced fat losses by using homogenized milk is well known for unconcentrated milk (Metzger and Mistry 1994; Nair *et al.* 2000) and is attributed to the modified fat globule membrane. It is to be stressed, that the amount of permeate deducted from the cheesemaking experiments due to microfiltration was not considered in the determination of the presented values. When taking into consideration that permeate contains almost no fat, the presented fat content decreases if dividing the fat content of the whey through the corresponding value of i.

Table 8.4: Effect of homogenization and microfiltration on the composition of raw cheese and whey. The presented values are means of at least duplicate measurements.

	Standard[1] n.h., i=1	n.h., i=1.98	8MPa, i=1	8MPa, i=1.24	8MPa, i=1.82	8MPa, i=1.93
Curd						
Dry Matter (%)	24.77±0.25	37.64	25.05	25.56	30.89	33.57
Raw Cheese						
Dry Matter (%)	50.46±0.61	55,40	49.18	49.15	55.02	53.17
Protein (%)	23.00±0.44	26,40	22.66	24.11	26.00	24.21
Fat (%)	23.52±0.96	25,98	23.93	23.98	25.86	25.48
Whey						
Dry Matter (%)	6.47±0.19	7.17	6.44	6.37	7.06	6.61
Protein (%)	0.74±0.09	1.04	0.70	0.86	1.17	1.03
Fat (%)	0.15±0.04	0.64	0.10	0.19	0.22	0.18

[1]presented values are means of three independent cheesemaking experiments; i: concentration factor of MF; n.h.: not homogenized

Summarizing up the results, it is concluded that curd treatment time should be fitted to the dry matter content of each milk system and that homogenization of milk prior to MF is promising since fat losses are diminished.

8.3.1.4 Dry Matter, Fat and Protein Recovery

The passage of constituents from milk into the raw cheese is presented in Table 8.5. The actual recoveries of dry matter, protein and fat generally increased with increasing concentration factor. Fat recovery in cheese has been reported to improve by the use of homogenized milk and cream (Peters 1956; Metzger and Mistry 1994) and similar trends were also seen in this experiment (Table 8.5). For instance, the adjusted fat recovery of homogenized, unconcentrated milk increased by 2 % compared to the standard and by 7 % compared to 2-fold concentrated, unhomogenized milk. This is coherent with the low fat losses in whey presented in Table 8.4. Protein recovery likewise increased and this may be attributed to the effect

of covering the secondary fat globule membrane with casein and whey protein due to homogenization. The homogenized fat globules may behave to some extent like large casein micelles (Buchheim 1986) and are incorporated into the casein network. Theoretically, more casein particles participate in the network, thus reducing the amount in the effluent whey. However, the effect of MF also contributes to the increased protein recovery as likewise reported St-Gelais *et al.* (1995) for Cheddar cheese manufacture. The increase of fat and protein recovery reflected on the dry matter recovery as well, whereby the increase was smaller.

Table 8.5: Effect of homogenization and microfiltration on dry matter, protein and fat recovery in the raw cheese.

	Standard[1] n.h., i=1	n.h., i=1.98	8MPa, i=1	8MPa, i=1.24	8MPa, i=1.82	8MPa, i=1.93
DM RC (%)	50.6±0.9	64,6	50.4	55.2	67.1	69.6
ad RC DM (%)	50.6±0.9	46,6	50.4	50.7	53.0	51.5
Protein RC (%)	79.2±1.3	87.3	82.2	84.8	89.3	91.1
ad RC Protein (%)	79.2±1.3	78.0	82.2	82.3	83.6	85.4
Fat RC (%)	90.9±2.3	85,4	92.9	96.7	97.2	96.0
ad RC Fat (%)	90.9±2.3	85,5	92.9	96.8	97.3	96.0

[1]presented values are means of three independent cheesemaking experiments; ad RC: adjusted recovery calculated from equation 8.2; DM: dry matter; i: concentration factor of MF; n.h.: not homogenized; RC: recovery

8.3.1.5 Yield of Raw Cheese

Concerning the economic background of cheesemaking, it is essential to give a statement about the proportion of inputs and outputs. This was taken care of by calculating the adjusted actual cheese yield, Y_a*. A comparison between yield of cheese made from concentrated and unconcentrated milk is yet possible and influences of factors may be yet revealed. Following Guinee *et al.* (2006), an attempt to assign potential differences in cheese yield to the direct effect of treatment per se rather than to intertreatment differences associated with milk composition (levels of fat or protein) or cheese composition (moisture), cheese yield was expressed in a number of formats as defined earlier (eq. 8.3 to 8.6).

Yield of raw cheeses calculated on different approaches are given in Table 8.6. Actual cheese yield (Y_a) increased with a rise in concentration factor of MF. This is consistent with the increasing actual recovery of the individual components found in Table 8.5 and with results reported for Cheddar cheese (Neocleous *et al.* 2002a;

Guinee *et al.* 2006). Y_a* of unhomogenized retentate (n.h., i = 1.98) decreased and this is explainable by component losses in whey, by formation of curd fines and in particular by the high dry matter content of the raw cheese. The positive effect of the interaction of homogenization and MF on the recoveries are reflected in Y_a* as well, but are less pronounced if retentates were highly concentrated (i > 1.8). This is explainable by the high dry matter content of the raw cheese (Table 8.4) causing a decrease in cheese weight. If this difference in raw cheese moisture is eliminated by using the moisture-adjusted yield, Y_{ma} strongly increases with increasing concentration factor of MF. Furthermore, if fat, protein and moisture are normalized to reference contents of the standard, Y_{mafpam} of raw cheese made from homogenized and concentrated milk increased with a rise in i if compared to the standard.

Table 8.6: Effect of homogenization and microfiltration on yield of raw cheese.

	Standard[1] n.h., i=1	n.h., i=1.98	8MPa, i=1	8MPa, i=1.24	8MPa, i=1.82	8MPa, i=1.93
Y_a (%)	11.56±0.13	19.63	11.90	14.52	20.35	20.78
Y_a* (%)	11.56±0.13	9.91	11.90	11.71	10.94	10.77
Y_{afpam} (%)	11.56±0.13	10.51	11.92	11.92	11.34	11.35
Y_{ma} (%)	11.56±0.13	21.55	11.60	14.14	22.19	21.90
Y_{mafpam} (%)	11.56±0.13	11.54	11.62	11.61	12.36	11.96

[1]presented values are means of three independent cheesemaking experiments; n.h.: not homogenized; raw cheese yield expressions: Y_a = actual yield (kg raw cheese/100 kg of milk); Y_a* = adjusted actual yield; Y_{afpam} = yield per 100 kg of milk, normalized to reference fat (2.96 %) and protein (3.34 %) levels; Y_{ma} = moisture-adjusted yield; Y_{mafpam} = moisture-adjusted (49.54 %) yield per 100 kg of milk normalized to reference fat and protein levels

Regarding the different calculation procedures presented in Table 8.6, Y_{mafpam} is recommended for yield calculation since it considers both, moisture content of the cheese and differences in cheese milk composition. If this is taken into account homogenization and MF increases yield in tendency.

8.3.1.6 Functional Properties of Ripened Cheese

The cheese composition along with functional properties of cheese after four weeks of ripening are given in Table 8.7. The cheeses corresponded to standards for Gouda cheese (van den Berg *et al.* 2004). Texture analysis revealed that the samples did not fracture (D_B) if cheeses were made from concentrated milks, whether homogenized prior to MF or not.

The F_B-values measured at 66 % of deformation increased with increasing concentration factor and were almost 2 to 3-fold the value of the standard. In a non-representative degustation, this effect was detected as well. The effect of increased hardness (F_B) and impaired fracturability (D_B) may be rather attributed to the raised dry matter of the cheeses made from concentrated milks than to the effect of MF. However, an effect of MF on the texture profile may be discussed since St-Gelais *et al.* (1995) and Neocleous *et al.* (2002b) found a relationship between hardness and increasing protein and fat contents due to MF. The F_B- and F_D-values of cheese made from homogenized, unconcentrated milk were slightly lower compared to the standard and this is consistent with results of Metzger and Mistry (1994) who reported a reduction in hardness for Cheddar cheese if cream was homogenized prior to cheesemaking.

The results indicate, that if curd treatment is not fit to the individual concentration factor, dry matter of curd, raw cheese and ripened cheese increases compared to the standard, hence, altering the texture profile of the cheeses.

Table 8.7: Effect of homogenization and microfiltration on composition and functional properties of cheese after four weeks of ripening. The presented values are means of at least duplicate measurements.

	Standard[1] n.h., i=1	n.h., i=1.98	8MPa, i=1	8MPa, i=1.24	8MPa, i=1.82	8MPa, i=1.93
Dry Matter (%)	56.0±1.2	60.34	56.24	56.49	59.52	57.95
FDM (%)	44.88±0.61	45.21	45.47	45.36	44.91	47.33
MNFS (%)	58.76±0.95	54.54	58.79	58.50	55.25	57.94
Texture						
F_B in N	17.2±7.2	41.37	13.45	41.88	59.71	26.42
D_B in %	50.08	> 66	49	> 66	> 66	> 66
F_D in N	9.4±2.3	9.05	8.65	11.81	17.92	7.29
Yellow Index	37.5±1.5	38.73	35.18	29.82	29.52	35.57
Meltability (CI)	5.05±0.55	3.5	1.08	1.54	1.50	1.21
Proteolysis	15.76±0.85	14.20	15.36	n.d.	n.d.	13.38

[1]presented values are means of three independent cheesemaking experiments; CI: circle index; D_B: deformation at fracture; FDM: fat-in-dry-matter; F_B: peak force at which the sample fractures; F_D: force at 33 % deformation; MNFS: moisture in non-fat solids; n.d.: not determined; n.h.: not homogenized

Cheese made from homogenized milk, whether concentrated or not, had a lower yellow index (Y_i), whereas MF of unhomogenized milk had no effect on Y_i. Additionally, the L-value, corresponding to whiteness, decreased (data not shown).

This reported Lemay *et al.* (1994) for Cheddar cheese and Rudan *et al.* (1998) for Mozzarella cheese as well.

The effect of homogenization was also remarkable on the meltability of cheese samples. Meltability decreased in the order: standard > MF concentrate made from unhomogenized milk > MF concentrate made from homogenized milk > homogenized milk. Jana and Upadhyay (1992) and Tunick *et al.* (1993) found a decrease in meltability for Mozzarella cheese as well, whereas Nair *et al.* (2000) reported an increase in meltability for Cheddar cheese. No influence on meltability of Mozzarella produced from homogenized cream reported Poduval and Mistry (1999). The decrease in meltability of cheese might be attributed to a delayed breakdown of casein, especially if bearing in mind that the casein/chymosin ratio increased with increasing i.

To evaluate the effect of casein/chymosin ratio on meltability and proteolysis, cheese was manufactured in another experiment (data not shown) from homogenized 1.5-fold concentrated milk and the casein/chymosin ratio was varied. If the casein/chymosin ratio was adjusted to the value of the standard, proteolysis increased but meltability was unaffected. This indicates that the structure of the casein-fat-network contributes more to the diminished meltability than proteolysis and consequently MF. These results seem to be connected with results of Steffl (1999), who found no relationship between UF and a decrease in proteolysis and meltability of soft cheese, respectively, but stated a markedly decrease in meltability, if whey proteins in cheese milk were heat-denatured to a degree > 95 % prior to 6-fold UF and further cheesemaking. Although, no experiments were conducted on homogenized milk, some conclusions from her observations may be drawn.

Due to covalent bond-formation between β-lactoglobulin and κ- and α_{s2}-casein during heating, “cross-link” casein polymers (Lelievre and Lawrence 1988) are derived. These polymers are incorporated into the casein matrix and, since unaffected during ripening, hinder the cheese sample to spread during the meltability test, hence, low meltability is observed. Furthermore, the heating temperature was supposed to be insufficient to crack these high energy bonds (> 330 kJ/mol). No high-energy bonds result from homogenization, but a large number of casein-fat-particles are obtained, interacting with casein during rennet-induced gel formation. The fat globules are yet

incorporated into the cheese matrix, like the “cross link” casein polymers, loosing its inert filler function and furthermore its property to flow while temperature increases.

Figure 8.3 demonstrates another effect of homogenization on cheese functionality; the reduction of oil release during heating/melting. Homogenization of milk (Peters 1956; Jana and Upadhyay 1992; Tunick 1994) and cream (Metzger and Mistry 1995) reduced the free oil in Cheddar as well as in Mozzarella cheeses. This reduction in free oil may be a result of improved emulsification of the fat by adsorbed casein and partly whey protein on the fat globule surface due to homogenization. The latter may contribute to impaired meltability and oiling off by formation of covalent disulfide bonds during heating, additionally stabilizing the network and therefore diminishes oiling off. Heating those samples decrease the degree of oiling off as is shown in Figure 8.3.

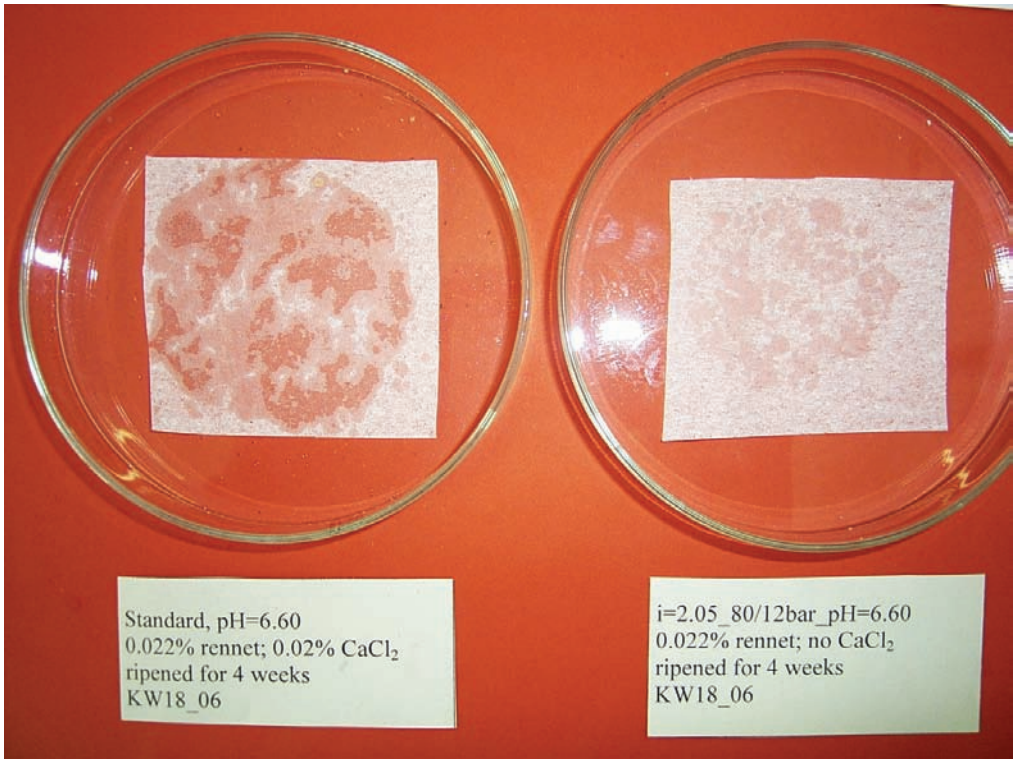


Figure 8.3: Oil release of a cheese sample made from unhomogenized (left side) and made from homogenized 2-fold concentrated milk (right side) as detected after removing the melted samples.

8.3.2 Series B

Considering the results gained in series A, it was essential to modify the cheesemaking procedure to overcome problems like too high dry matter content of raw and ripened cheese.

8.3.2.1 Milk Composition

The standard milk was processed as previously described to obtain homogenized and unhomogenized milks of different protein and fat levels due to microfiltration. The mean composition of the different milk systems is given in Table 8.8. With increasing concentration factor, contents of calcium, dry matter, fat and protein increased.

Table 8.8: Composition of processed milks used in semi-hard cheese manufacture.

	Standard[1] n.h., i=1	n.h., i=2.02	8MPa, i=1.24	8MPa, i=1.51	8MPa, i=1.76	8MPa, i=2.05
Dry Matter (%)	11.20±0.20	17.05±0.04	12.91±0.08	14.29±0.07	15.77±0.05	17.92±0.04
Protein (%)	3.28±0.05	6.25±0.06	3.81±0.01	4.49±0.05	5.14±0.04	6.27±0.12
Fat (%)	2.96±0.06	5.90±0.00	3.60±0.00	4.37±0.06	5.17±0.06	6.18±0.03
Calcium (%)	0.118±0.002	n.d.	0.143±0.003	0.164±0.001	0.184±0.001	0.213±0.001

[1]presented values are means of three independent cheesemaking experiments; i: concentration factor of MF; n.d.: not determined; n.h.: not homogenized

8.3.2.2 Semi-Hard Cheese Manufacture

From the results and experiences gained in series A, it becomes obvious that the cheesemaking procedure should be modified in order to save material and time and to improve cheese quality. The procedure is depicted in Figure 8.4 and the modified steps are black coloured. The unmodified steps are grey coloured. The modifications are explained in the following:

The total process, including ripening, coagulation and curd treatment, was performed at a constant temperature of 35 °C. The temperature was chosen because fermentation can also be done at 35 °C and warming-up during curd treatment becomes superfluously (no scale-up problem). In addition, the syneresis experiments in Chapter 7 were carried out at 35 °C and provide useful data to design the modified process. From these experiments, equation 8.7 was generated and allows to estimate the curd treatment time as affected by protein concentration and pH.

$$t(DM_{45\,min}) = 21.94 - 27.54 \times i - 4.68 \times pH + 4.63 \times i^2 - 6.26 \times (i \times p_1) \qquad (8.7)$$

i: concentration factor of MF; $t(DM_{45min})$: time in min to reach the dry matter of curd grains made from standard milk after 45 min of curd treatment

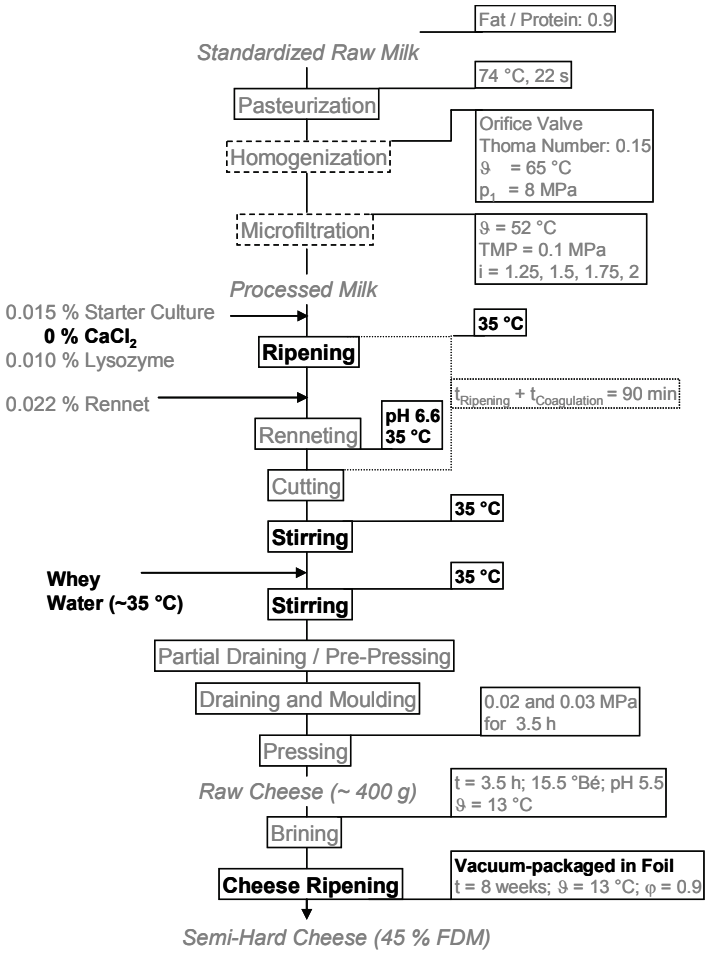


Figure 8.4: Flow sheet for the modified cheesemaking procedure. The modified steps are black coloured against the unmodified grey coloured steps. (: optional; FDM: fat-in-dry-matter; i: concentration factor of MF; p_1: homogenization pressure; t: time; TMP: transmembrane pressure)

Since equation 8.7 uses the coded values, i.e. -1, 0, 1 for i = 1, 1.5, 2 and pH 6.6, 6.5, 6.4, the equation was transferred for the practical application to equation 8.8.

$$t(DM_{45\,min}) = -176.75 - 98.12 \times i + 46.8 \times pH + 18.52 \times i^2 - 3.13 \times (i \times p_1) + 4.695 \times p_1 \quad (8.8)$$

A process temperature of 35 °C is favourable when considering that the pH drop in retentates due to microbial fermentation is delayed, since the buffer capacity increases with increasing protein content. Preliminary experiments indicated that bacterial activity, and thus acidification, increases if the temperature is raised from 30 °C to 35 °C (data not shown).

No calcium was added to the concentrated milks, since results of texture analyses demonstrated that with the exception of i = 1.24, equal or even higher curd firmness,

defined as the peak force during the compression cycle (Bourne 1978), was reached after 60 min of coagulation if compared to values of the standard (Figure 8.5).

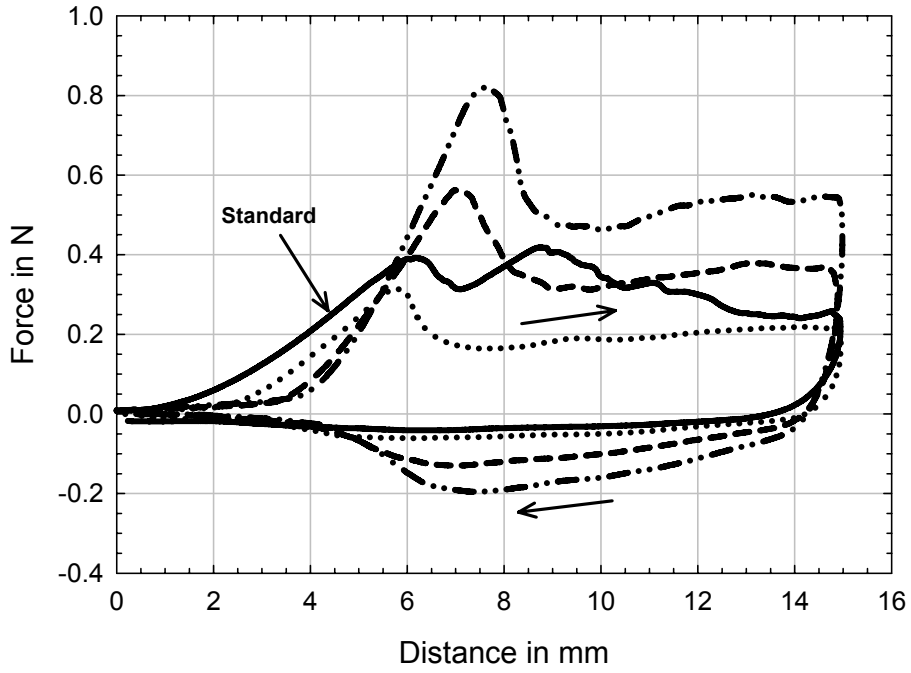


Figure 8.5: Force-distance curves of gels made from different milks adjusted to pH 6.6 after 60 min of coagulation. Curd firmness is defined as the peak force during the compression cycle. Each curve represents values of five individual measurements. (— : standard milk with added calcium and coagulated at 30 °C; ••••• : 8MPa, i = 1.24 without calcium addition and coagulated at 35 °C; – – – – : 8MPa, i = 1.51 without calcium addition and coagulated at 35 °C; – • • – : 8MPa, i = 1.76 without calcium addition and coagulated at 35 °C)

This may be attributed to the increase of coagulation temperature (5 °C) and the higher calcium contents of the retentates compared to the standard (Table 8.8). Nájera *et al.* (2003) likewise reported faster curd firming when coagulation temperature was increased, since fusion of micelles within the strands proceeds faster, hence, leading to higher bond formation per time (Zoon *et al.* 1988b).

If the calculation of whey drainage prior to the addition of water considers the permeate release during MF, whey drainage above i = 1.43 is obsolete. For i < 1.43 the weight of whey to be drained, $m_{Whey\ to\ Drain}$, can be calculated following equation 8.9 (further details are given in the Appendix).

$$m_{Whey\ to\ Drain} = m_{Re\ tentate} \times (1 - 0.7 \times i) \qquad i < 1.43 \qquad (8.9)$$

i: concentration factor of MF; $m_{Retentate}$: weight of retentate used for cheese manufacture

Below i = 1.43, the weight of water, m_{Water}, to be added is obtained by equation 8.10.

$$m_{Water} = m_{Retentate} \times i \times 0.2 \qquad i < 1.43 \qquad (8.10)$$

Above i = 1.43, the amount of water to be added, m_{Water}, is calculated by equation 8.11 (further details are given in the Appendix).

$$m_{Water} = m_{Retentate} \times 0.286 \qquad (8.11)$$

The cheese was vacuum-packaged in ripening foil to conserve the composition of the raw cheese. This helps to prevent uneven ripening conditions (like in series A) and overlapping of factors like high or low moisture content on the individual functionality tests.

Following this procedure, the basic parameters applied in the individual cheesemaking trials as affected by milk treatment are presented in Table 8.9. Less rennet and culture were added to concentrated milks compared to the standard as likewise described in series A. CT strongly decreased with increasing protein concentration, although no calcium was added. This was expected as discussed before. In contrast to series A, the starter-to-drain-time decreased with increasing protein concentration, since curd treatment time was adapted. It is to be noted that in the case of 2-fold concentrated milks the calculated curd treatment time was prolonged up to 10 min, since cutting of the curd in the cheese vat was difficult and syneresis was therefore delayed.

Table 8.9: Effect of homogenization and microfiltration on cheesemaking parameters.

	Standard n.h., i=1	n.h., i=2.02	8MPa, i=1.24	8MPa, i=1.51	8MPa, i=1.76	8MPa, i=2.05
Calcium (%)[f]	0.020	0	0	0	0	0
Rennet (%)[f]	0.022	0.011	0.018	0.015	0.013	0.011
Culture (%)[f]	0.015	0.007	0.012	0.010	0.009	0.007
$\vartheta_{Process}$ (°C)	30-39	35	35	35	35	35
RT (min)	30	60	52	63	66	68
CT (min)[*]	60	30	38	27	24	22
CTT (min)	45	3	43	30	17	6
S-to-D (min)	135	93	133	120	107	96
Whey (%)[**]	-30	0	-12.5	0	0	0
Water (%)	20	28.6	25	28.6	28.6	28.6

CTT: curd treatment time was calculated according to equation 8.8; i: concentration factor of MF; n.h.: not homogenized; S-to-D: starter-to-drain time; ϑ: temperature; [f]calculation of the concentration was performed by considering the original volume of milk before MF; [*]cutting time (CT) after rennet addition was determined using low-amplitude strain oscillation rheometry; [**]drained whey before water addition

8.3.2.3 Composition of Curd, Raw Cheese and Whey

The compositions of curd, raw cheese and whey are shown in Table 8.10. The presented values for the standard are means of three independent cheesemaking experiments and considering the low standard deviations, reproducibility of cheesemaking was given. Furthermore, the compositions of curd, raw cheese and whey of series A (Table 8.4) and B considering the standard were comparable. This was expected since the composition of the standard cheese milk was likewise comparable (Table 8.1).

In comparison to series A, the dry matter of the curd at the end of curd treatment was rather homogenous among the different trials and close to the value of the standard. In consequence, the dry matter content of the raw cheeses was likewise in the same range. Dry matter, fat and protein losses in the effluent whey during curd treatment only increased for the 2-fold concentrated retentate of unhomogenized milk, whereas homogenization prior to MF slightly reduced losses up to i = 1.5. Like in series A, it is to be stressed, that the amount of permeate deducted from the cheesemaking experiments due to microfiltration was not considered in the determination of the presented values. Since permeate contains almost no fat, this would mean an additional decrease in the presented fat content that may be estimated by dividing the fat content of the whey by the corresponding value of i.

Table 8.10: Effect of homogenization and microfiltration on the composition of raw cheese and whey. The presented values are means of at least duplicate measurements.

	Standard[1] n.h., i=1	n.h., i=2.02	8MPa, i=1.24	8MPa, i=1.51	8MPa, i=1.76	8MPa, i=2.05
Curd						
Dry Matter (%)	24.38±1.51	26.34	25.70	24.80	24.33	23.38
Raw Cheese						
Dry Matter (%)	47.77±1.53	44.82	46.50	48.02	48.48	47.48
Protein (%)	22.30±0.72	21.43	21.33	21.80	21.55	21.86
Fat (%)	22.39±0.72	20.97	21.56	22.28	23.89	22.81
Whey						
Dry Matter (%)	6.68±0.11	7.50	6.59	5.94	6.24	6.79
Protein (%)	0.75±0.13	1.25	0.73	0.74	0.87	0.96
Fat (%)	0.17±0.01	0.69	0.09	0.07	0.07	0.15

[1]presented values are means of three independent cheesemaking experiments; i: concentration factor of MF; n.h.: not homogenized

Considering the aim to reach a comparable dry matter content prior to the draining and pre-pressing step, it is concluded, that the modified cheese manufacture procedure is promising even for highly concentrated retentates. Below i = 1.25, modification of the cheesemaking procedure is not necessary, since trials in series A and B were comparable.

8.3.2.4 Dry Matter, Fat and Protein Recovery

The recovery of the components in raw cheese is presented in Table 8.11. Apart from protein recovery, the recoveries of series A and B considering the standard were comparable, highlighting again that cheese manufacture was reproducible. The actual recoveries generally increased with increasing protein and fat level of the cheese milks.

In comparison to series A (Table 8.5), the same effect of the interaction of homogenization and microfiltration on the recoveries were observed, but the adjusted recoveries (ad RC) were even higher (Table 8.11). This is consistent with the lower component losses with whey (Table 8.10) and may be attributed to the shorter curd treatment time. In contradiction to series A (Table 8.5), the recoveries of 2-fold concentrated, unhomogenized milk were yet comparable with the recoveries of the standard (Table 8.11). This indicates, that the modified manufacture procedure improves cheesemaking efficiency of highly concentrated, unhomogenized milk as well.

Table 8.11: Effect of homogenization and microfiltration on dry matter, protein and fat recovery in the raw cheese.

	Standard[1] n.h., i=1	n.h., i=2.02	8MPa, i=1.24	8MPa, i=1.51	8MPa, i=1.76	8MPa, i=2.05
DM RC (%)	51.5±1.1	67.6	57.8	63.2	68.8	70.9
ad RC DM (%)	51.5±1.1	51.4	52.6	52.6	53.6	56.2
Protein RC (%)	82.0±1.0	88.0	89.5	91.3	93.6	92.7
ad RC Protein (%)	82.0±1.0	82.6	85.4	84.8	84.7	86.0
Fat RC (%)	91.9±0.6	91.0	95.5	95.6	99.3	98.1
ad RC Fat (%)	91.9±0.6	91.0	95.6	96.0	100.0	97.6

[1]presented values are means of three independent cheesemaking experiments; ad RC: adjusted recovery was calculated from equation 8.2; DM: dry matter; i: concentration factor of MF; n.h.: not homogenized; RC: recovery

8.3.2.5 Yields of Raw Cheese

Figure 8.6 illustrates that cheese was successfully produced from homogenized and microfiltered milk.

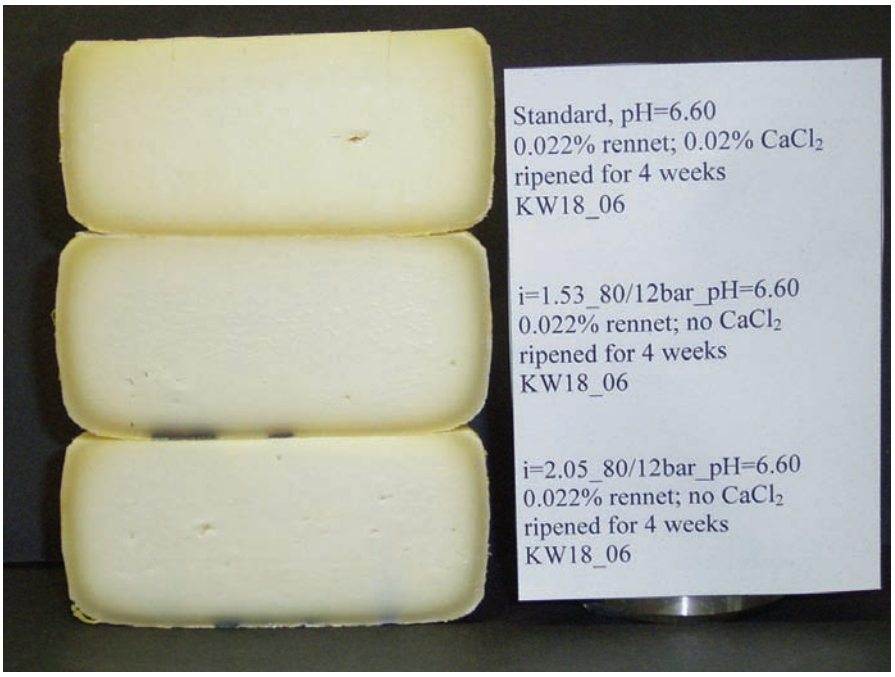


Figure 8.6: Semi-hard cheeses made from differently treated milk after four weeks of ripening.

Yield of raw cheese is given in Table 8.12. Y_a increased with a rise in contents of protein and fat and this is consistent with the increasing actual recoveries of the individual components found in Table 8.11.

Table 8.12: Effect of homogenization and microfiltration on yield of raw cheese.

	Standard[1] n.h., i=1	n.h., i=2.02	8MPa, i=1.24	8MPa, i=1.51	8MPa, i=1.76	8MPa, i=2.05
Y_a (%)	12.04±0.06	25.71	16.06	18.82	22.37	27.47
Y_a* (%)	12.04±0.06	12.75	12.95	12.54	12.57	13.05
Y_{afpam} (%)	12.04±0.06	13.20	13.52	13.26	13.54	13.77
Y_{ma} (%)	12.04±0.06	24.12	15.63	18.92	22.70	27.30
Y_{mafpam} (%)	12.04±0.06	12.38	13.16	13.33	13.74	13.67

[1]presented values are means of three independent cheesemaking experiments; n.h.: not homogenized; raw cheese yield expressions: Y_a = actual yield (kg raw cheese/100 kg of milk); Y_a* = adjusted actual yield; Y_{afpam} = yield per 100 kg of milk, normalized to reference fat (2.96 %) and protein (3.28 %) levels; Y_{ma} = moisture-adjusted yield; Y_{mafpam} = moisture-adjusted (52.23 %) yield per 100 kg of milk normalized to reference fat and protein levels

The effect of the interaction of homogenization and microfiltration on Y_a and Y_a* depicts Figure 8.7.

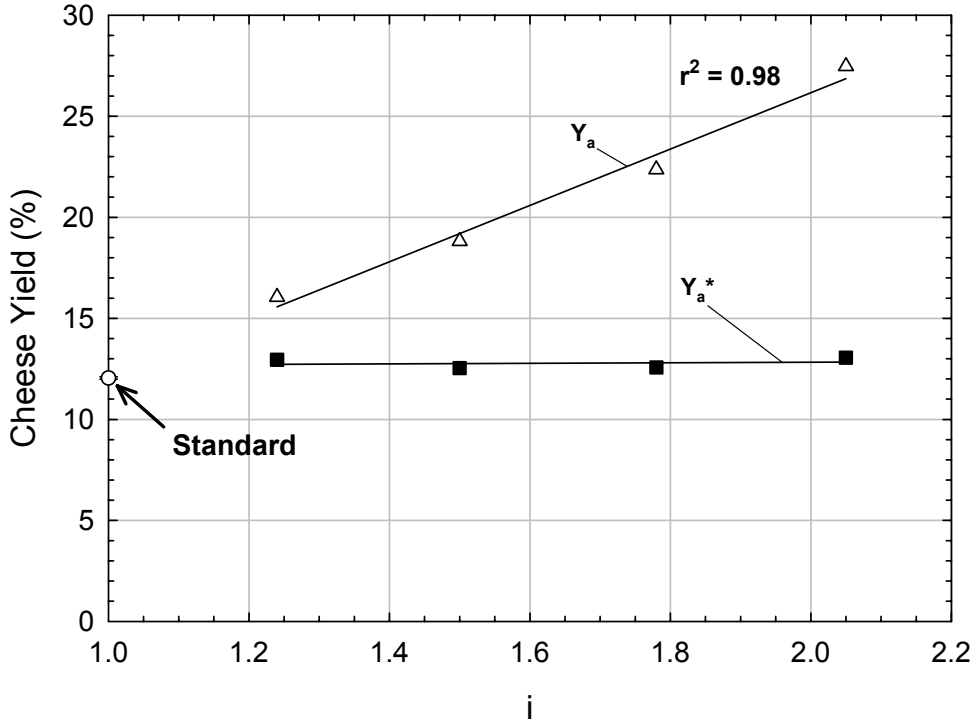


Figure 8.7: Relationship between concentration factor of MF (i) and the actual yield (Y_a: Δ), respectively the adjusted actual yield (Y_a*: ■), of raw cheeses made from different milks, as described in Table 8.8.´

Y_a increases linearly with increasing protein levels and this is in agreement with findings of Guinee *et al.* (2006). In contradiction to series A (Table 8.6) and due to the modified cheese manufacture procedure, Y_a* slighty increases with an increase in i, demonstrating good cheesemaking efficiency. If the difference in cheese moisture is eliminated, Y_{mafpam} increased up to 1.5 % compared to standard (Table 8.12). In comparison, series B reveals higher cheese yield than series A.

8.3.2.6 Functional Properties of Ripened Cheese

The comparison of series A and B reveals the influence of conditions of ripening on cheese composition after four weeks of ripening (Table 8.7 and 8.13). Cheeses in series A were coated and ripened naturally, whereas cheese in series B, apart from the 1.93-fold concentrated trial, was vacuum-packaged and foil-ripened. Cheese in series A and cheese of the 1.93-fold concentrated trial lost more moisture and the contents of dry matter and MNFS therefore increased compared to the trials in series B. The composition and individual functional properties of the standard cheese in series A and B were thus not comparable. Cheeses in series A corresponded to

standards for Gouda cheese (van den Berg *et al.* 2004), whereas cheeses in series B did not.

Table 8.13: Effect of homogenization and microfiltration on composition and functional properties of cheese after four weeks of ripening. The presented values are means of at least duplicate measurements.

	Standard[1] n.h., i=1	n.h., i=2.02	8MPa, i=1.24	8MPa, i=1.51	8MPa, i=1.76	8MPa[#], i=2.05
Dry Matter (%)	50.34±0.89	47.59	48.02	49.70	50.55	57.13
Calcium (%)	0.66	n.d.	0.62	0.62	0.66	0.73
FDM (%)	45.11±0.89	44.74	44.63	45.71	46.32	43.94
MNFS (%)	64.23±0.75	66.60	66.20	65.10	64.60	57.20
Texture						
F_B in N	9.96±0.62	9.32	9.01	10.37	10.52	21.60
D_B in %	43.99±1.80	26.27	34.20	40.15	36.07	43.90
F_D in N	8.24±0.99	8.50	8.68	9.34	10.28	18.05
Yellow Index	29.29±1.52	25.33	20.49	23.65	21.75	31.33
Meltability (CI)	8.91±1.40	10.00	1.44	2.69	1.88	3.00
Proteolysis	15.50±3.23	14.86	10.94	12.87	12.63	13.32

[1]presented values are means of three independent cheesemaking experiments; CI: circle index; D_B: deformation at fracture; F_B: peak force at which the sample fractures; F_D: force at 33 % deformation; FDM: fat-in-dry-matter; MNFS: moisture in non-fat solids; n.d.: not determined; n.h.: not homogenized; [#]cheese was not vacuum-packaged during ripening

The modified cheesemaking procedure allows to produce cheese with comparable dry matter content. The strong influence of dry matter content in series A on cheese texture was therefore prevented and no influence of milk processing on cheese texture is interpretable from the results presented in Table 8.13. The low D_B value measured for the unhomogenized, 2-fold concentrated milk is explainable by a low cheese pH of 4.80 resulting from further acidification during ripening due to the high moisture content. This should be avoided in further studies and experiments should be carried out to proof the result even under natural conditions of cheese ripening. Although, no calcium was added to the concentrated milks, the amount of calcium in the ripened cheese was comparable to the standard as expected from preliminary results.

Similar effects of homogenization on meltability and yellow index were principally observed. Figure 8.8 reflects another result yet not discussed.

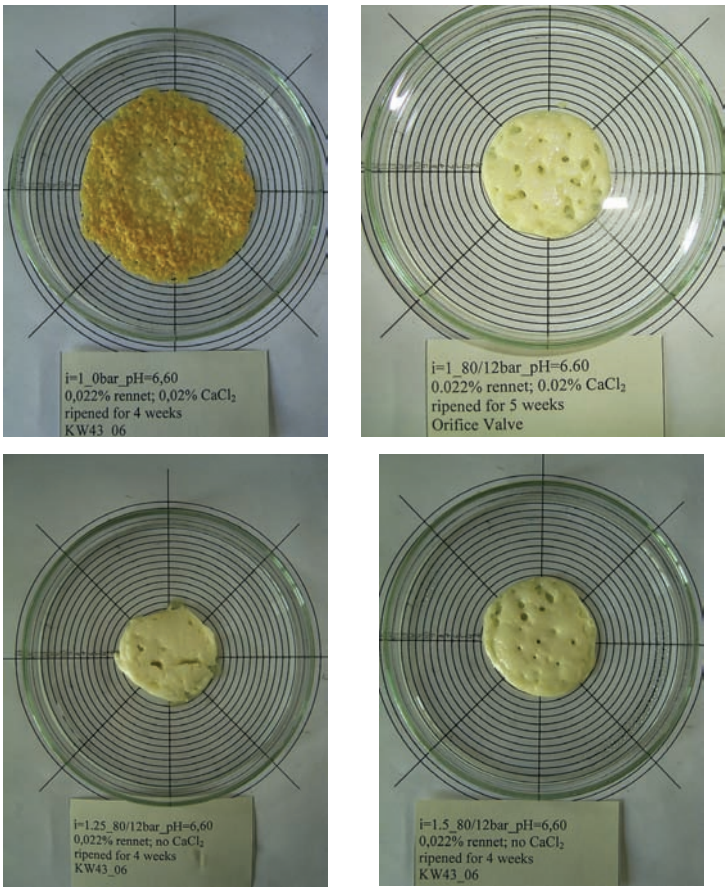


Figure 8.8: Melt spreads and colour of semi-hard cheese samples manufactured from differently treated milk systems, obtained by the covered Schreiber test.

Samples of cheese made from homogenized milk, whether afterwards concentrated or not, showed no or only little browning after heating compared to the standard. L*a*b-values were determined for the melted samples and the results reflect the visual impression. The yellow index of melted samples made from homogenized milk was in the range of 30 that corresponds to the value measured for the unmelted cheeses. In contradiction, yellow index of the melted standard cheese sample was about 70.

No effect of homogenization on lactose and further degradation to galactose is found in the literature, since the molecular size of sugars is too small to be influenced by low-pressure homogenization. The observed result may be rather attributed to the reduced meltability that reduces heat transfer, since the height of the sample remained almost unchanged. Furthermore, a skin resulted from drying out of the surface of the sample, additionally reducing the heat transfer. A further explanation for a reduced Maillard reaction and browning, respectively, may be the reduction in free water in the cheese due to homogenization. The homogenized fat globules are

covered with proteins that interact with the serum phase and water binding increases. Less free water, necessary for the Maillard reaction, is available, hence, browning decreases.

Little effect of MF is observed on functional properties of the cheese if the manufacture procedure is modified, whereas the effect of homogenization is still clearly noticeable.

8.4 Conclusion

The main objective of the feasibility study was to investigate the influence of increasing milk fat and protein levels due to MF and homogenization prior to MF on cheese yield and functional properties of semi-hard cheese. Furthermore, results of preliminary experiments indicated that the cheesemaking procedure usually applied should be modified. Therefore, two series of experiments, A and B, were undertaken in succession to evaluate the influence of modifications in cheesemaking procedure. In series A, cheese was manufactured following a conventional procedure. From the experiences gained by series A, the cheesemaking procedure in series B was modified and simplified.

(i) The adjusted recoveries found for unhomogenized MF-concentrated milk decreased or were comparable to the standard. In contradiction, homogenization and the interaction of homogenization and MF increased the adjusted protein and fat recovery, respectively, to cheese by 3 to 6 % and by 2 to 5 %, respectively. If the values were not adjusted to the original milk volume, the recoveries, especially for the dry matter, even increased.

(ii) Actual cheese yield (Y_a) markedly increased by 3 to 14 % within both series with increasing protein and fat contents of the cheese milk. This effect was even intensified if the milk was homogenized prior to MF, since the fat and protein recoveries increased. A linear relationship between Y_a and concentration factor of MF (i) was found for retentates of homogenized milk.

(iii) If the cheese yield in series A was adjusted to the original milk volume prior to MF (Y_a*), the interaction of MF and homogenization did not improve the cheese yield compared to the standard. Similar results were found for the

moisture-adjusted cheese yield (Y_{mafpam}). This results from the excessive curd treatment, leading to losses of curd fines and to high dry matter contents in the curd. In consequence, these cheeses were harder and did not fracture.

(iv) These problems were overcome by modifications of the cheesemaking procedure (series B). Compositions of curd, raw cheese and ripened cheese among the individual trials were yet comparable. In consequence, texture properties among the cheese trials were comparable and the moisture-adjusted cheese yield increased by 1 to 1.5 % compared to the standard and to trials of series A, respectively.

(v) Due to the modified manufacture procedure, MF had little effect on cheese functionality, whereby homogenization still markedly altered cheese colour, and decreased cheese meltability, browning during heating and oiling off.

(vi) The modified cheese manufacture procedure has several advantages towards the conventional procedure, as follows: a constant process temperature of 35 °C was applied and with increasing i, significant amounts of additives (calcium, rennet and lysozyme) were saved. Processing time decreased with increasing i due to a shorter curd treatment time.

By means of a simplified cheesemaking procedure several problems were overcome considering the use of MF retentates in semi-hard cheesemaking. Furthermore, it was shown that the interaction of homogenization and MF increases adjusted cheese yield and component recovery. Hence, a cheese maker would obtain more amount of cheese from the same amount of original milk, that is a key factor to remain competitive in the production of commodity-type cheeses such as Gouda.

8.5 Acknowledgment

This research project was supported by the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn), the AiF and the Ministry of Economics and Technology. AiF-Project No.: 14073N. I acknowledge kindly the technical assistance of Luc Mertz and Giovanni Migliore and the persistent help of Regina Schuster-Wolff-Bühring and Philipp Schenkel.

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9 CONCLUDING REMARKS

9.1 Rennet Curd and Cheese Texture as influenced by Homogenization

The effect of homogenization on rennet-induced gel formation, curd texture, cheese composition and functional properties was previously discussed in detail. In this chapter, an attempt is made to link the various observations by an approach based on pictures obtained from confocal laser scanning microscopy, rheological data and results of meltability.

Figure 9.1 depicts the microstructure of curd from standard milk (1), homogenized milk (2) and homogenized, microfiltered milk (3). They had comparable gel strengths since coagulation time was adapted. 1A clearly illustrates the influence of the large unhomogenized fat globules on the curd structure. The globules that appear as black holes are surrounded by coherent protein strands (coded in red) leading to a honeycombed structure. In 2A and 3A, the protein structure is more difficult to interpret since the black holes are much smaller. The magnification is generally too low to give a statement whether the protein strands in 1A are different in thickness compared to the strands in 2A and 3A. The effect of homogenization on fat globule size is markedly demonstrated. The unhomogenized fat globules (coded in green) in 1B are much larger than those in 2B and 3B. Merging both pictures (C) indicates that the protein/fat-network of curd in 2C is denser than of curd in 1C due to the number of small fat particles that are homogenously emulsified and dispersed in the matrix. The compactness of the network is even more pronounced if the contents of casein and homogenized fat globules are increased due to MF as can be seen in 3C.

It was shown in Chapter 6, that the curd firming rate (CFR) of homogenized milk was higher compared to unhomogenized milk and this result is yet explainable. Approximately 75 % of the secondary milk fat globule membrane after homogenization is covered with casein and fragments of casein (Cano-Ruiz and Richter 1997). Hence, the large number of fat globules in curd 2 and 3 behave to some extent like large casein micelles and participate during enzymic coagulation (Buchheim 1986). Homogenization increases the number of structure-forming particles per volume and furthermore reduces steric stabilization by decreasing the energy barrier as discussed in detail in Chapter 6. Consequently, the probability of aggregation of casein and casein-fat particles is higher compared to unhomogenized

milk. Thus, faster bond formation appears that provides higher CFR values if small non-destructive deformation measurement is applied.

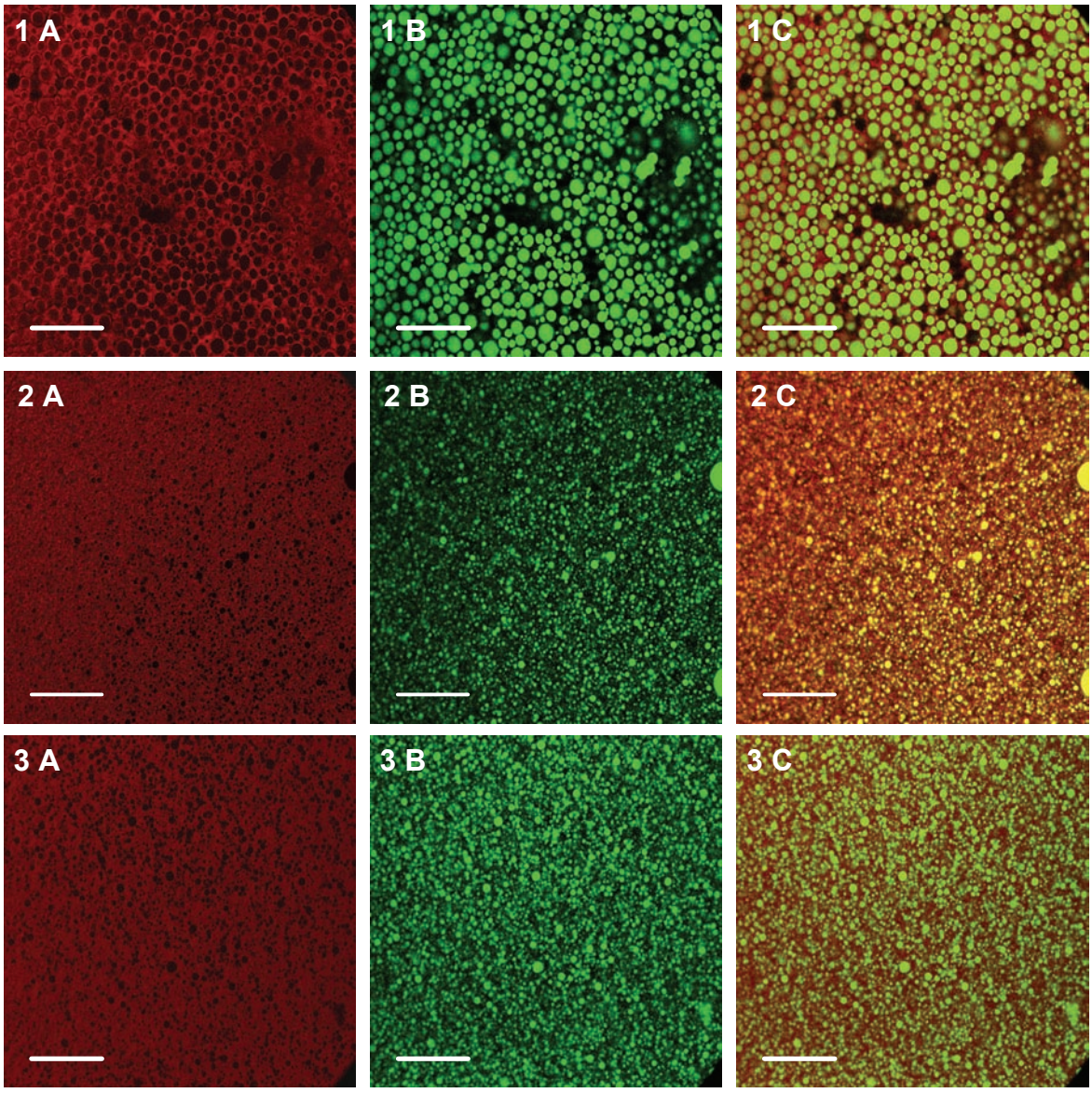

Figure 9.1: Confocal laser scanning micrographs of curd made from (1) standard milk, (2) homogenized milk (8 MPa) and (3) homogenized milk (8 MPa) prior to 2-fold MF. Coagulation time was 60 min for (1) and (2), and 30 min for (3). A: protein is coded in red; B: fat is coded in green; C: picture A and B are merged. Samples were prepared following the description given in the Appendix. Level of magnification: 63-fold; bar 26 µm.

Based on these results, a model is presented to discuss the interactions of casein micelles and fat globules during rennet-induced gel formation as affected by homogenization of milk (Figure 9.2). The contents of casein and fat are in both, A and B, similar. In A, the unhomogenized fat globules act as inert fillers, i.e. they do not contribute to the gel matrix that exclusively exists of casein micelles. It may be

assumed, that the fat globules act as breakers of the casein network, but results by Lopez *et al.* (2007) demonstrate that the fat globules are smaller in size than the pores of the casein network. The model gel reveals a porous structure in which the spherical milk fat globules are entrapped. In contradiction, the homogenized fat globules in B, much smaller in size and covered with casein and casein fragments, now contribute to the network. Bond formation yet occurs between covered fat particles and casein, between fat particles and fat particles and casein and casein as well, resulting in a compact gel with many non-covalent cross links.

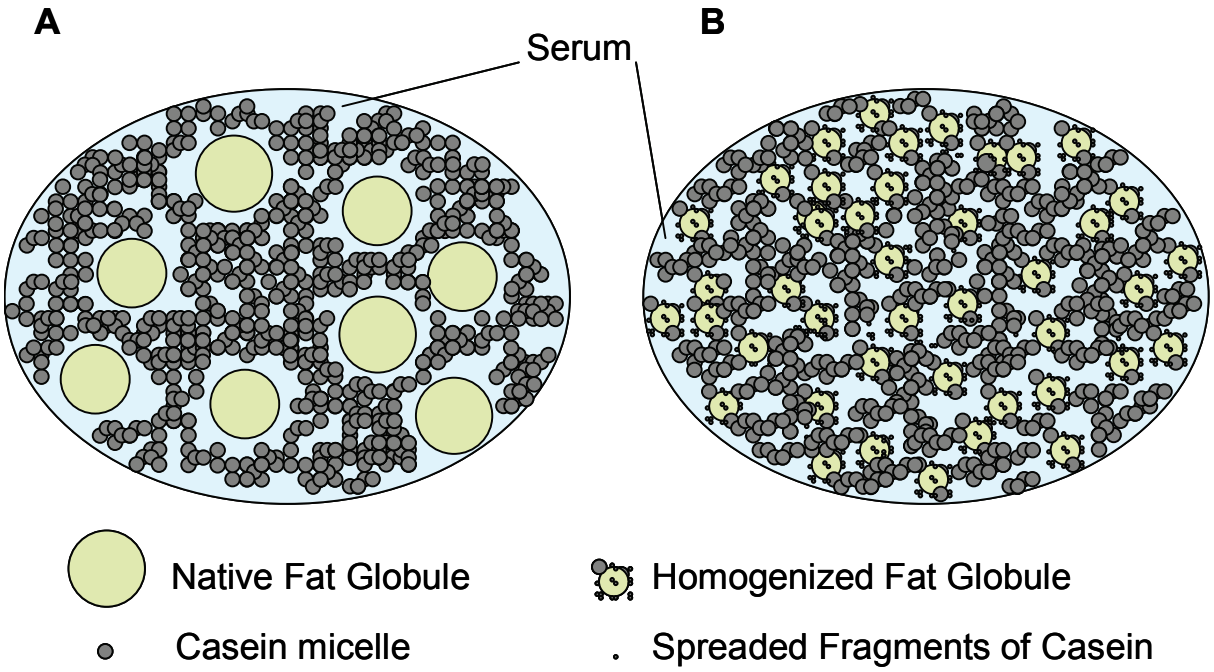

Figure 9.2: Schematic representation of the interactions of casein micelles and fat globules during rennet-induced gel formation as affected by homogenization of milk. A: unhomogenized milk; B: homogenized milk.

Although the gel of a homogenized milk seems to be more compact, curd firmness (F-60 value) obtained by a destructive compression method (Chapter 6 and 7) was lower compared to the milk gel of unhomogenized milk. This is confusing if considering the filled gel composite model according to Visser (1991). Very briefly, he stated that increasing the gel and decreasing the filler volume fraction will increase gel firmness. As was previously shown, homogenization increases the gel volume and decreases the filler volume, since fat globules are yet incorporated into the network. One explanation may be that due to its incorporation, the casein-fat network may evade if mechanical stress is applied, hence, lowering the resistance of the gel.

Furthermore, pictures obtained by scanning electron microscopy showed that in comparison to untreated milk the protein-fat strands of microfluidized milk (very intensive homogenization) were bulky and of uneven thickness and apparently more strands ended in nodules that were not tied into the gel structure (Tosh and Dalgleish 1998). Further experiments, like scanning electron microscopy, should be carried out to get a better insight into gel microstructure and its effect on rheological measurements.

Figure 9.3 depicts the microstructure of ripened cheese depending on milk treatment. We would like to point out, that the level of magnification is 25 compared to 63 in Figure 9.1, so that structure elements appear smaller.

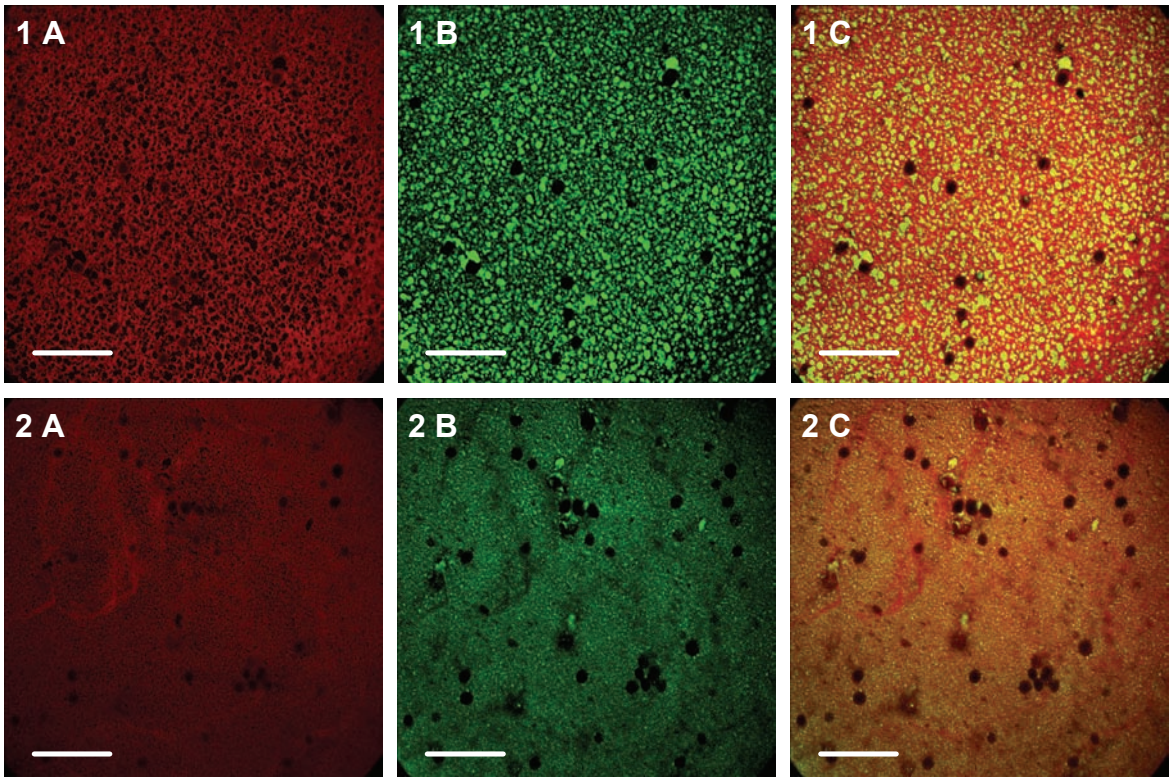

Figure 9.3: Confocal laser scanning micrographs of cheese made from (1) standard milk (ripened for 10 weeks) and (2) homogenized milk (8 MPa) prior to 1.5-fold MF (ripened for 9 weeks). A: protein is coded in red; B: fat is coded in green; C: picture A and B are merged. Black holes in the pictures correspond to carbon dioxide bubbles generated during ripening from microbial fermentation. Samples were prepared following the description given in the Appendix. Level of magnification: 25-fold; bar 105 µm.

Product 1 represents cheese that was manufactured from standard milk, whereas product 2 was made from homogenized, concentrated milk (8 MPa, i = 1.5). Like in Figure 9.1, the unhomogenized green coloured fat globules in 1B are much larger

than those in 2B. Unlike product 2, more irregularly shaped fat globules appear in product 1 (C), probably caused by disruption due to enzymatic hydrolysis of the native globule membrane and distortion by protein rearrangements due to proteolysis during ripening (Lopez *et al.* 2007). In Chapter 8, it was shown that meltability decreased if milk was homogenized prior to cheesemaking and that MF did only slightly affect meltability. Results concerning proteolysis were comparable among the samples. If bearing in mind that homogenization increases the number of bonds within the network (Figure 9.2) and that the secondary milk fat globule membrane is quite stable against microbial and enzymatic deterioration, it may be assumed, that the fat is protected against oiling off and furthermore a higher degree of proteolysis is necessary to generate meltability comparable to the standard.

9.2 The Economic Potential of the combined Application of Homogenization and Microfiltration (MF) in Cheese Manufacture

Although cheesemaking is an ancient art, implementation of innovative technologies is becoming increasingly necessary to remain competitive in the production of semi-hard cheeses. Besides other factors influencing cheese manufacture and efficiency, this work dealt in particular with the study of combining two innovative technologies, microfiltration (MF) and homogenization via an orifice valve, to evaluate their effects on cheesemaking and functional properties. Since homogenization and MF are expensive technologies, knowledge about their economic feasibility is important if implemented in cheesemaking.

Unlike ultrafiltration (UF), MF is yet not widely applied in cheese manufacture, although this membrane filtration process is not new in the cheese industry. Problems like fouling of MF membranes were the main reason for the lack of attention to MF (Papadatos *et al.* 2003), but recent developments have claimed that MF prior to cheesemaking will become a widely used method in the near future (Maubois 2002; Mistry and Maubois 2004). Furthermore, unlike cheese milk standardization by the addition of milk protein powders, MF only concentrates the components of the original milk system, hence, MF may be implemented for standard cheese manufacture without any legal registrations. Papadatos *et al.* (2003) compared conventional Cheddar and Mozzarella cheesemaking with a cheese

manufacture procedure using 2-fold MF concentrated skim milk. They considered the values of the coproducts like MF-permeate as well. Table 9.1 represents the costs for conventional and MF Cheddar cheese production (Papadatos *et al.* 2003). The cost of MF cheddar was estimated to be 20.65 cents per kg of cheese. This is lower than the production cost of the same plant using the same volume of unfortified raw milk (28.20 cents per kg of cheese).

Table 9.1: Average manufacturing costs for conventional Cheddar cheese, not including the cost of raw milk and estimates of average manufacturing costs for Cheddar cheese when 2x microfiltration (MF) is used (Papadatos *et al.* 2003).

	Conventional cheesemaking (100 kg of unconcentrated milk in cheese vat)			2x MF prior to cheesemaking (100 kg of 2x MF retentate in cheese vat)	
Cost item	Cost per kg of cheese[1] (cents)	Percentage of total costs	Change in cost when 2x MF is used relative to conventional[2]	New cost per kg of cheese (cents)	Percentage of total costs
Labour					
Supervisory	0.84	3.0	50 % decrease	0.42	2.0
Direct fixed	1.01	3.6	50 % decrease	0.51	2.5
Direct variable	9.79	34.7	35 % decrease	6.42	31.1
Total labour	11.65	41.3		7.35	35.6
Capital costs					
Depreciation/interest	3.88	13.8	20 % decrease	3.12	15.1
Utilities					
Electricity	0.34	1.2	30 % decrease	0.24	1.1
Fuel	2.03	7.2	30 % decrease	1.42	6.9
Sewage	0.17	0.6	30 % decrease	0.12	0.6
Total utilities	2.53	9.0		1.77	8.8
Materials					
Laboratory	0.17	0.6	50 % decrease	0.08	0.4
Production	4.90	17.3	25 % decrease	3.68	17.8
Packaging	2.03	7.2	0 % decrease	2.03	9.8
Cleaning	0.84	3.0	50 % decrease	0.42	2.0
Total materials	7.94	28.1		6.21	30.0
Repair and maintenance	0.34	1.2		0.34	1.6
Property tax/insurance	1.18	4.2	0 % decrease	1.18	5.7
Production inventory	0.34	1.2	0 % decrease	0.34	1.6
Other expenses	0.34	1.2	0 % decrease	0.34	1.6
TOTAL	28.20	100		20.65	100

[1]The average cost per kg of cheese corresponds to plants with a capacity of 440,000 kg of milk per day, operating 21 h per day, and six days per week. [2]Based on plant utilization.

Furthermore, they demonstrate that the cost per mass of cheese for the MF Cheddar is similar to the production cost for a conventional plant of Cheddar with double capacity running unfortified raw milk (21.11 cents per kg of cheese).

In summary, MF cheesemaking exhibited lower cost of cheese production than conventional cheesemaking, but the MF Cheddar plant had higher total manufacture cost because of the additional cost of MF of skim milk. However, the benefit in net revenue from MF was higher (€ 1.94 per 100 kg raw milk for Cheddar) than the difference in manufacturing costs (€ 0.23 per 100 kg raw milk). A further benefit was addressed to MF cheesemaking through improved plant efficiency. Although, MF is not supposed to increase cheese yield efficiency (i.e., more cheese from the same amount of unconcentrated milk), because no increase in retention of serum proteins in the cheese produced from MF retentate is expected (Neocleus *et al.* 2002), the results of Papadatos *et al.* (2003) remarkably demonstrate its economic potential in cheesemaking.

Little information is found in the literature about the costs of homogenization if applied in cheese manufacture. However, energy costs thereby incurred can be estimated following equation 9.1.

$$P_{elect.} \sim P_{mech.} = \Delta p \times \dot{V} \qquad (9.1)$$

$P_{elect.}$: electric power; $P_{mech.}$: mechanical power; p: homogenization pressure; $\dot{V}$: volume flow of milk

Furthermore, homogenization increases the cheese yield, which is an important and significant advantage for the industry, as reported in Cheddar and Chanco cheeses (Metzger and Mistry 1994; Nair *et al*. 2000; Brito *et al.* 2006) and from our results. The greater yields are attributed to the smaller losses of fat in the whey released during the elaboration, as well as to the greater moisture content shown by these cheeses.

When taking these significant economic advantages of each technology for granted, the combination of both should even improve the advantages for the industry and this hypotheses, among others, was proofed in the previous chapters. It was demonstrated, that if the conventional manufacture procedure was changed to the simplified protocol (series B), cheese yield efficiency, expressed as moisture and component adjusted yield (Y_{mafpam}), increased by 1 to 1.5 % towards the standard. Assuming a German cheese factory with a production of 2000 tons per year, 20 tons more cheese from the same amount of milk may be obtained. Concurrently, it was demonstrated that the amounts of additives (calcium, rennet, starter culture)

decreased with increasing concentration factor, e.g. for a 1.5-fold concentrated milk no calcium and only 70 % of the original rennet volume were added. Hence, costs for additives decrease. These advantages are obvious and if the economic comparison of Papadatos *et al.* (2003) is taken into consideration even more.

9.3 Further Need for Research

Along with the outstanding economic advantages discussed in 9.2, the analysis of cheese properties showed some peculiarities that are mainly based on the effect of homogenization on rennet gel and cheese structure, as shown in 9.1. The question, whether the changed cheese properties, e.g. meltability, are perceived as negative or positive, addresses on the individual point of view of the cheese manufacturer and consumer as well. However, a few hypothesis shall be stated that should be ascertained by further research.

The studies of Poduval and Mistry (1999), and Nair *et al.* (2000) indicate that homogenization of cream results in an unchanged meltability of Mozzarella or even increased meltability of Cheddar cheese. This may be attributed to clustering of the homogenized fat globules during homogenization of cream with fat contents > 20 %, as pictures obtained from scanning electron microscopy indicated (Metzger and Mistry 1995). These clusters are reported to be stable and are even not disrupted by gently agitation. It may be assumed, that these clusters are incorporated into the casein matrix causing irregularities within the network, enhancing therefore oiling off and improved meltability during heating. Hence, further experiments may be carried out with the addition of homogenized cream.

As an alternative for the addition of homogenized cream, homogenized MF retentate may be used for cheesemaking. In this case, the order of processing is changed, i.e. MF of the cheese milk is carried out prior to homogenization. First results obtained from following this procedure (data not presented) showed for concentration factors of MF (i) above 1.75 an improved homogenization efficiency, i.e. particle sizes obtained at comparable pressures decreased compared to homogenized, unconcentrated milk. As a consequence, less energy is needed to achieve comparable particle sizes and additionally, depending on i, less milk volume is to be homogenized compared to unconcentrated milk.

Another attempt to improve meltability, and therefore induces changes in curd and cheese structure, is supposed to result from heating of the MF retentates prior to cheesemaking. Results of Schreiber and Hinrichs (2000) showed that with rising concentration of casein due to MF of skim milk, more whey proteins could be denatured by still allowing gel formation and good cheesemaking properties, i.e. for ~2-fold MF of skim milk (corresponds to 6 % casein) 8 mg whey proteins/1000 mg retentate may be denatured to achieve a gel strength of pasteurized skim milk (1 mg/1000 mg milk). The idea would be to block a certain amount of κ-casein due to covalent bond formation between β-lactoglobulin and whey proteins, respectively, hence decreasing the amount of available links within the network. Furthermore, Vasbinder (2002) presented a model which describes the influence of pH on formation of β-lg-β-lg interactions and/or κ-casein-β-lg interactions occurring in milk during heat treatment for 10 min at 80 °C (Figure 9.4). Remarkable differences in rennet-induced gel formation were observed, indicating that both, pH and whey protein denaturation, are measures to influence gel structure. Thus, the contribution of homogenized fat globules to the network formation may be equalled or at least be influenced. Further research is necessary to link this result to the investigated milk treatments and to estimate its effect on cheese manufacture and quality.

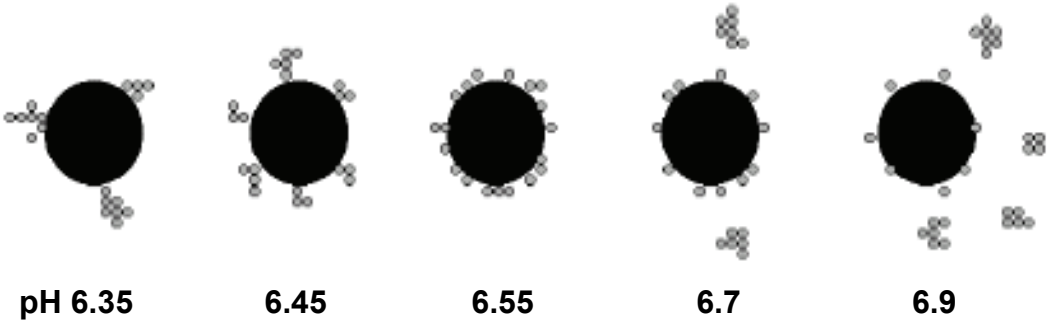

Figure 9.4: A schematic representation of the interactions between casein micelles and whey proteins occurring in milk during heat treatment for 10 min at 80 °C at pH values ranging from 6.35 to 6.9 (Vasbinder 2002). The small circles represent denatured whey proteins, the large circles the casein micelles. The whey proteins are either present in aggregates or covalently associated with the casein micelle. Native whey proteins are not included in the figure.

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10 SUMMARY

Although cheesemaking is an ancient art, modern cheese production relies on the implementation of innovative technology and tailor-made starter bacteria to remain competitive in the production of commodity-type cheeses such as soft and semi-hard cheese. Any intervention in the cheesemaking procedure, i.e. in milk composition, milk treatment and microbial fermentation, affects textural properties of curd at cutting and finally syneresis. The latter is the key step in cheesemaking since the degree of syneresis determines the moisture content of the raw cheese, by which ripening as well as rheological properties and sensory are affected.

This work aimed to investigate the syneresis of rennet curd grains in order to generate a kinetic model for predicting syneresis. On the one hand, the experiments covered the implementation of EPS-(exopolysaccharide producing) cultures in the manufacture of soft cheese and likewise the investigation of the cheesemaking potential of Dahlem Cashmere goat's milk. On the other hand, the interrelated effects of homogenization, microfiltration and pH on rheological properties of rennet-induced milk gels, on syneresis and finally on cheese composition, yield and functionality were to study.

Standardized, pasteurized bovine milk, pasteurized bovine skim milk and pasteurized skim milk of Dahlem Cashmere and German White goats were used for the experiments. Homogenization was carried out at 65 °C applying pressures up to 12 MPa using an orifice valve provided by the University of Karlsruhe. Microfiltration up to 2.5-fold was carried out using a pilot-scale membrane processing unit. Low-amplitude strain oscillation rheometry was used to study the viscoelastic properties of rennet-induced milk gels. Firmness of rennet-type gels and consistency were evaluated by means of a texture analyzer. Syneresis of rennet curd grains was followed with a model system close to cheesemaking conditions. In order to calculate cheese yield and recovery of milk components as influenced by composition of cheese milk and milk treatment, all products and by-products were analyzed using standard methods.

Fermentation media inoculated with non-EPS-producing *Streptococcus thermophilus* and EPS-producing strains of *Lactococcus lactis* subsp. *cremoris* and *Lactobacillus sakei* were added in a concentration from 5 % to 10 % (w/w) to the milk prior to soft

cheese manufacture. The cheesemaking experiments showed that the addition of fermentation media with EPS-cultures retarded syneresis, accelerated microbial fermentation and finally caused ripening problems. By means of model experiments regarding syneresis and influence of pH value, the manufacture of soft cheese was technologically adapted. The approach demonstrated that soft cheese manufacture was yet feasible and moisture content of the raw cheese was increased by the addition of fermentation media, inoculated with EPS-cultures.

Three mathematical models were compared for their suitability describing syneresis and providing kinetic parameters. The kinetic parameters obtained by a linearised model gave best curve fittings to the experimental data with high coefficient of correlation ($r^2 > 0.99$). Therefore, the model is recommended for calculation of kinetic data. Furthermore, the model provides a parameter (RWR_{max}) that gives information about the endpoint of syneresis. From this value, interpretation about the curd structure and the interaction of milk composition and physical factors on syneresis is possible.

The strategy to evaluate cheesemaking properties of milk from different breeds and species of ruminant demonstrated the superior cheesemaking properties of Dahlem Cashmere (DC) goat's milk compared to German White goat's milk. Curd firmness of DC milk was even higher at cutting than the gel made from bovine milk, promising a sufficient stability of the curd grains against the mechanical stress applied during curd treatment in semi-hard cheese production. The syneresis experiments revealed that DC and bovine milk were comparable regarding RWR_{max}. Curd grains made from DC milk rapidly released whey during curd treatment, leading to a shorter curd treatment time compared to bovine milk. Thus, DC milk is favourable for cheesemaking, even under conditions applied in semi-hard cheese manufacture using bovine milk.

The investigation of the impact of homogenization, microfiltration (MF), pH and their interaction on rheological properties of rennet-induced milk gels revealed that pH had the most important influence on rennet coagulation time, while curd firming rate and cutting time were strongly influenced by MF, pH and the interaction of MF and pH. Results of texture analysis confirmed these observations, whereas an increase in homogenization pressure strongly decreased curd firmness. This was not observed using oscillatory rheometry, so that cutting time prediction for homogenized milk

turned out to be difficult. It is assumed, that in the case of homogenization not only the number of bonds at cutting time determines curd firmness, but also the integration and distribution of the fat globules in the casein network.

Analysis of variance revealed that syneresis was significantly affected by homogenization, MF and pH. It was shown that milk composition and MF markedly influenced the endpoint of syneresis, RWR_{max}. Curd grains made from skim milk had the highest RWR_{max} value. It is assumed, that differences in curd microstructure due to fat globule distribution and content affect syneresis since cutting was performed at equal curd firmness. The experiments demonstrate that homogenization and MF can be combined to reach curd firmness and syneresis which are in accordance with values in conventional cheesemaking.

Combination of homogenization and MF was promising on cheese yield, and based on the results and experience gained in this study, a new and simplified process for semi-hard cheesemaking was invented. It was shown, that the adjusted cheese yield and component recovery increased due to the interaction of homogenization and MF.

The work showed that several factors clearly altered textural properties of curd and syneresis resulting in different cheese composition. A strategy was presented to overcome problems in cheese manufacture that demonstrated how to adapt process parameters. In particular, the combination of homogenization and MF in cheese manufacture is promising. Next steps should be to upscale the process in order to confirm the results even under production scale. Furthermore, the techno-functional properties of the cheese showed some interesting peculiarities that are mainly based on the homogenized fat globules being incorporated by rennet-induced gelation into the cheese structure. How to adjust the techno-functionality of the cheese should be ascertained by further research.

11 ZUSAMMENFASSUNG

Obwohl das Herstellen von Käse ein sehr altes Gewerbe ist, ist das Implementieren von innovativer Technologie und maßgeschneiderter Starterbakterien notwendig, um in der modernen Herstellung von Weich- und Schnittkäse wettbewerbsfähig zu bleiben. Jeglicher Eingriff in den Käsungsprozess, d.h. in die Zusammensetzung und Behandlung der Milch, sowie in fermentative Vorgänge, beeinflusst die Textureigenschaften der Labgele und letzten Endes die Synärese. Letztere ist der wesentliche Prozessschritt bei der Käseherstellung, da der Synäresegrad den Serumgehalt im Rohkäse bestimmt, wodurch der Reifungsprozess sowie die rheologischen und sensorischen Eigenschaften beeinflusst werden.

Die Arbeit hatte zum Ziel, die Synärese von Bruchwürfeln zu verfolgen, um ein kinetisches Modell zu generieren, anhand dessen die Synärese vorhersagbar wird. Einerseits waren die Experimente auf das Einbringen von EPS- (Exopolysaccharid-bildende) Kulturen in die Weichkäseherstellung sowie auf die Untersuchung des Potenzials von Dahlem Cashmere Ziegenmilch für die Käseherstellung ausgelegt. Andererseits waren die in Wechselbeziehung stehenden Effekte von Homogenisieren, Mikrofiltrieren und pH auf die rheologischen Eigenschaften von labinduzierten Milchgelen, auf die Synärese und schließlich auf die Käsezusammensetzung, -ausbeute und Funktionalität zu untersuchen.

Standardisierte, pasteurisierte Kuhmilch, pasteurisierte bovine Magermilch und pasteurisierte Magermilch von Dahlem Cashmere Ziege und Deutscher Weißen Edelziege wurden für die Experimente eingesetzt. Die Milch wurde mit einer Lochblende bei 65 °C und Drücken von bis zu 12 MPa homogenisiert und in einer Pilotmembrananlage bis zu 2.5-fach konzentriert bzw. mikrofiltriert. Die viskoelastischen Eigenschaften labinduzierter Milchgele wurden durch oszillatorische Rheometrie bestimmt. Die Festigkeit und Konsistenz der Labgele wurden mit einem Texturprüfgerät ermittelt. Die Synärese von Bruchkörnern wurde unter käsereiüblichen Bedingungen mit einem Modellsystem verfolgt. Um die Käseausbeute und Rückhaltung von Milchinhaltsstoffen zu berechnen, wurden alle Produkte und Nebenprodukte über Standardmethoden analysiert.

Weichkäse wurde mit *Streptococcus thermophilus* und EPS-bildenden Stämmen von *Lactococcus lactis* subsp. *cremoris* und *Lactobacillus sakei* produziert. Die EPS-

Bildner wurden in einem Medium angezogen und der Käsereimilch in Konzentrationen von 5 und 10 % (w/w) zugegeben. Die Zugabe von EPS-Medium verzögerte die Synärese und erhöhte die mikrobielle Fermentation, so dass Reifungsprobleme auftraten. Mit Hilfe von Modellversuchen bezüglich der Synärese und des pH Wertes wurde die Weichkäseherstellung technologisch angepasst. Die Vorgehensweise zeigte, dass die Weichkäseherstellung nun möglich und der Serumgehalt der Rohkäse durch den Zusatz von EPS-Medium erhöht war.

Drei mathematische Modelle wurden auf ihre Eignung, die Synärese zu beschreiben, verglichen. Die kinetischen Parameter, die über ein linearisiertes Modell berechnet wurden, erzielten die beste Kurvenanpassung mit hohem Korrelationskoeffizient ($r^2 > 0.99$), so dass dieses für die Berechnung der kinetischen Daten zu empfehlen ist. Darüber hinaus bietet das Modell mit dem Parameter RWR_{max} Informationen bezüglich des Maximalwerts der Synärese. Über diesen Wert können Aussagen getroffen werden, inwiefern die Struktur des Labgels und das Zusammenspiel von Milchzusammensetzung und physikalischer Faktoren die Synärese beeinflussen.

Die Strategie zur Evaluierung der Käsereieigenschaften von Milch verschiedener Wiederkäuerzüchtungen und -arten veranschaulichte die hervorragenden Eigenschaften von Dahlem Cashmere (DC) Ziegenmilch im Vergleich zu Milch Deutscher Weißer Edelziegen. Die Festigkeit der Gele aus DC Milch war zum Zeitpunkt des Schneidens sogar höher als die aus Kuhmilch, so dass von einer ausreichenden Stabilität der Bruchkörner gegenüber den mechanischen Kräften auszugehen ist, welche während der Bruchbearbeitung in der Schnittkäseherstellung angewandt werden. Die Synäreseversuche zeigten, dass die RWR_{max} Werte von DC Milch und Kuhmilch vergleichbar waren. Die Synärese von Bruchkörnern aus DC Milch war stark beschleunigt, so dass eine kürzere Bruchbearbeitungszeit gegenüber Kuhmilch resultierte. Das Verkäsen von DC Milch ist somit auch unter Bedingungen günstig, welche in der Herstellung von Schnittkäse aus Kuhmilch angewandt werden.

Die Untersuchung der Einflüsse von Homogenisieren, Mikrofiltrieren (MF), pH und ihr Zusammenspiel auf die rheologischen Eigenschaften von labinduzierten Milchgelen offenbarte, dass das pH die Gerinnungszeit am stärksten beeinflusste, während die Gelverfestigungsrate und der Schneidezeitpunkt deutlich durch das MF und pH, sowie deren Interaktion beeinflusst wurden. Ergebnisse der Texturanalyse bestätigten diese Beobachtungen, wohingegen eine Zunahme des

Homogenisierdrucks die Gelfestigkeit maßgeblich verringerte. Dies steht im Gegensatz zu Ergebnissen der oszillatorischen Rheometrie, so dass die Ermittlung des Schneidezeitpunktes im Fall von homogenisierter Milch problematisch ist. Es wird angenommen, dass im Falle des Homogenisierens nicht nur die Anzahl an Bindungen die Gelfestigkeit am Schneidezeitpunkt bestimmt, sondern auch die Einbindung und Verteilung der Fetttropfen in das Caseinnetzwerk.

Das Homogenisieren, MF und der pH-Wert beeinflussten die Synärese signifikant. Es wurde gezeigt, dass die Milchzusammensetzung und das MF den Endpunkt der Synärese, RWR_{max}, deutlich beeinflussten. Bruchkörner aus Magermilch hatten den höchsten RWR_{max} Wert. Da das Schneiden bei gleicher Gelfestigkeit erfolgte, wird angenommen, dass die Synärese maßgeblich durch die Verteilung und den Gehalt an Fetttropfen beeinflusst wird, woraus sich Unterschiede in der Mikrostruktur des Labgels ergeben. Die Versuche verdeutlichen, dass durch das Kombinieren von Homogenisierung und Mikrofiltration Gelfestigkeiten und Synäresewerte erzielt werden, die mit Werten aus der traditionellen Käseherstellung übereinstimmen.

Die Kombination aus Homogenisieren und anschließender MF war bezüglich der Käseausbeute viel versprechend und aus den Ergebnissen und Erfahrungen, die in dieser Arbeit erzielt wurden, wurde ein neuer und vereinfachter Prozess der Schnittkäseherstellung erstellt. Die auf die ursprüngliche Milchmenge bezogene Käseausbeute und Rückhaltung von Inhaltsstoffen nahmen daraufhin auf Grund der Interaktion von Homogenisieren und MF zu.

Die Arbeit verdeutlichte, dass mehrere Faktoren die Textureigenschaften der Labgele und die Synärese deutlich veränderten, wodurch sich Unterschiede in der Käsezusammensetzung ergaben. Um Probleme in der Käseherstellung zu überwinden, wurde eine Strategie vorgestellt, die aufzeigte, wie Prozessparameter anzupassen sind. Die Kombination aus Homogenisieren und MF offenbarte sich als besonders viel versprechend für die Käseherstellung. Anschließende Versuche sollten vorgenommen werden, um den Prozess auf industrielle Maßstäbe zu übertragen. Die techno-funktionellen Eigenschaften der Käse zeigten darüber hinaus interessante Besonderheiten, die sich v.a. aus der Integration der homogenisierten Fetttropfen in die Käsestruktur erklären lassen. Inwiefern sich die techno-funktionellen Eigenschaften gezielt beeinflussen lassen, sollte durch weitere Arbeiten beleuchtet werden.

12 APPENDIX

Chapter 8

Equation 8.9

According to the standard cheesemaking protocol, 30 % of whey are to be drained prior to the washing step (equation 11.1).

$$m_{\text{Whey to Drain}} = m_{\text{Milk}} \times 0.3 \tag{11.1}$$

m_{Milk}: weight of milk used for cheese manufacture; $m_{Whey\ to\ Drain}$: whey to be drained prior to water addition

If MF is applied the simple dependence is no longer valid since permeate is released during this process. Equation 8.9 and 11.5, respectively, were derived from equation 11.2 and 11.3. Equation 11.2 describes the whey drainage prior to the addition of water for retentate that amounts 30 % of the original weight of milk.

$$m_{\text{Whey to Drain}} = m_{\text{Re tentate}} \times i \times 0.3 \tag{11.2}$$

i: concentration factor of MF; $m_{Retentate}$: weight of retentate used for cheese manufacture

Equation 11.3 describes the whey, respectively, permeate release, $m_{Permeate}$, during MF depending on i.

$$m_{\text{Permeate}} = m_{\text{Re tentate}} \times (i - 1) \tag{11.3}$$

Since whey, respectively, permeate is partly released due to MF the appropriate quantity calculated by means of equation 11.3 is to be subtracted from the amount calculated by equation 11.2. This dependence leads to equation 11.4.

$$m_{\text{Whey to Drain}} = m_{\text{Re tentate}} \times i \times 0.3 - m_{\text{Re tentate}} \times (i - 1) \tag{11.4}$$

From the transformation of equation 11.4, equation 11.5 is obtained.

$$m_{\text{Whey to Drain}} = m_{\text{Re tentate}} \times (1 - 0.7 \times i) \tag{11.5}$$

The amount of $m_{Whey\ to\ Drain}$ becomes zero if i > 1.43. Hence, whey drainage above i = 1.43 is obsolete.

Equation 8.10

According to the standard cheesemaking protocol, 20 % of water with regard to the original weight of milk should be added after whey drainage. The effect of MF on whey drainage below i = 1.43 was equalled by equation 11.5 (eq. 8.9). Therefore, if whey drainage during cheesemaking is followed according to equation 11.5, equation 11.6, respectively 8.10, can be used for calculating the appropriate amount of water to be added.

$$m_{Water} = m_{Retentate} \times i \times 0.2 \qquad i \leq 1.43 \qquad (11.6)$$

$m_{Water:}$ the amount of water to be added

Equation 8.11

Following equation 11.5, whey drainage during cheesemaking above i = 1.43 is obsolete since equal or even higher amounts of permeate, respectively, whey are removed due to MF as demanded by the cheesemaking protocol. This dependence describes equation 11.7 in which factor W is equal or higher 1 below i = 1.43 and below 1 above i = 1.43. The latter indicates that more whey is removed by MF than demanded by the protocol. Therefore, equation 11.6 is to be multiplied with equation 11.7 to keep the ratio of whey to water constant.

$$W = \frac{m_{Retentate}}{m_{Retentate} \times i \times 0.7} \qquad (11.7)$$

In doing so, equation 11.8 is obtained.

$$m_{Water} = (m_{Retentate} \times i \times 0.2) \times \left(\frac{m_{Retentate}}{m_{Retentate} \times i \times 0.7} \right) \qquad (11.8)$$

From the transformation of equation 11.8, equation 11.9 is received.

$$m_{Water} = \frac{m_{Retentate} \times 0.2}{0.7} = m_{Retentate} \times 0.286 \qquad i > 1.43 \qquad (11.9)$$

Chapter 9

Confocal Laser Scanning Microscopy (CLSM)

Semi-hard cheese microstructure was examined during its manufacture and ripening using CLSM. The protein network was stained using Rhodamine B fluorescent dye (VWR International, Darmstadt, Germany). A lipid-soluble Nile Red fluorescent dye (Sigma–Aldrich, Munich, Germany) was used to label fat. Rhodamine B was dispersed in distilled water (0.01 g/L) and Nile Red in Polyethylene-Glycol 200 (Sigma–Aldrich, Munich, Germany; 0.02 g/L). The acrylic glass microscope slide used for the experiments had a cylindric hollow with a diameter of 25 mm and a depth of about 2 mm in its centre. The samples were covered with slips of 24 x 50 mm size and a thickness of 0.13 to 0.16 µm (Menzel Gläser, Braunschweig, Germany).

Milk preparation for examination of the curd microstructure was carried out as follows: calcium was added to standard milk in a concentration of 0.02 % (v/w) and pH adjustment to pH 6.5 was undertaken at 30 °C. No calcium was given to the retentate and pH adjustment to pH 6.5 was undertaken at 35 °C. Each dye was given to the milk in a concentration of 5 % (w/w) prior to renneting. For coagulation, 0.022 % (v/w) of rennet was added to the milk and retentate. After thoroughly mixing, 950 µL of the sample was transferred to the microscope slide and tempered in the dark at the appropriate temperature in an air-heated warming cupboard. Curd of standard milk and curd of retentate were analyzed after 60 min, respectively, 30 min of coagulation. These times correspond to approximately the cutting times determined in the rheometer.

Thin slices of ripened cheese, with a diameter of 25 mm and a thickness of about 2 mm, were prepared using a cheese borer and a sharp knife. Before transferring the cheese sample to the microscope slide, 38 µL of each staining solution was pipetted into the hollow. 38 µL of each staining solution was given onto the top of the sample and permit the diffusion of the stains for at least 2 hours in the dark at 4 °C.

Microstructural analyses were made using a confocal microscope (MRC-1024, Biorad, Hertfordshire, UK), which employed an argon/krypton laser in dual-beam fluorescent mode, with excitation wavelengths of 488 nm and 568 nm for fat and

protein, respectively. The intensity of the laser was set to 30 % and a Kalman filter (step 2) was used. The two-dimensional images had a resolution of 1024 x 1024 pixels and the pixel scale values were converted into micrometers using scaling factors of 0.51 µm and 0.20 µm per pixel at levels of magnification of 25 and 63, respectively. In the double-stained samples, the fat phase was coded in green and the protein phase was coded in red. Aqueous phase and any microscopic gas microbubbles in the slices may appear as black holes in the confocal micrographs.

The software Photoline32 (version 13.5, Computerinsel GmbH, Bad Gögging, Germany) was used to re-work the micrographs.

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