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MICROFILTRATION IN CHEESE AND WHEY PROCESSING

ANTTI HEINO

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CONTENTS

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ABSTRACT

Milk microfiltration (0.05-0.2 μm) is a membrane separation technique which divides milk components into casein-enriched and native whey fractions. Hitherto the effect of intensive microfiltration including a diafiltration step for both cheese and whey processing has not been studied.

The microfiltration performance of skimmed milk was studied with polymeric and ceramic MF membranes. The changes caused by decreased concentration of milk lactose, whey protein and ash content for cheese milk quality and ripening were studied. The effects of cheese milk modification on the milk coagulation properties, cheese recovery yield, cheese composition, ripening and sensory quality as well as on the whey recovery yield and composition by microfiltration were studied. The functional properties of whey protein concentrate from native whey were studied and the detailed composition of whey protein concentrate powders made from cheese wheys after cheese milk pretreatments such as high temperature heat treatment (HH), microfiltration (MF) and ultrafiltration (UF) were compared.

The studied polymeric spiral wound microfiltration membranes had 38.5% lower energy consumption, 30.1% higher retention of whey proteins to milk retentate and 81.9% lower permeate flux values compared to ceramic membranes. All studied microfiltration membranes were able to separate main whey proteins from skimmed milk. The optimal lactose content of Emmental cheese milk exceeded 3.2% and reduction of whey proteins and ash content of cheese milk with high concentration factor (CF) values increased the rate of cheese ripening. Reduction of whey protein content in cheese milk increased the concentration of caseinomacropeptide (CMP) of total proteins in cheese whey. Reduction of milk whey protein, lactose and ash content reduces milk rennet clotting time and increased the firmness of the coagulum. Cheese yield calculated from raw milk to cheese was lower with microfiltrated milks due to native whey production.

Amounts of α-lactalbumin (α-LA) and β-lactoglobulin (β-LG) were significantly higher in the reference whey, indicating that HH, MF and UF milk pretreatments decrease the amounts of these valuable whey proteins in whey. Even low CF values in milk microfiltration (CF 1.4) reduced nutritional value of cheese whey. From the point of view of utilization of milk components it would be beneficial if the amount of native whey and the CMP content of cheese whey could be maximized. Whey protein concentrate powders made of native whey had excellent functional properties and their detailed amino acid composition differed from those of cheese whey protein concentrate powders.

TIIVISTELMÄ

Maidon mikrosuodatus (0.05-0.2 μm) on kalvoerotustekniikka, joka jakaa maidon komponentit kaseiinikonsentraattiin ja natiiviin herajakeeseen. Tähän mennessä voimakasta mikrosuodatusta, joka sisältää diasuodatusvaiheen, ei ole tutkittu juuston ja heran prosessien kannalta.

Tässä tutkimuksessa tutkittiin rasvattoman maidon mikrosuodatusta polymeerisillä ja keraamisilla mikrosuodatuskalvoilla ja verrattiin kalvojen suorituskykyä. Mikrosuodatuksella alennetun juustomaidon laktoosin, heraproteiinin ja tuhkapitoisuuden vaikutuksia juuston laatuun ja kypsymiseen tutkittiin. Lisäksi tutkittiin mikrosuodatetun juustomaidon koostumuksen vaikutusta maidon juoksettumisominaisuuksiin, juustosaantoon, juuston koostumukseen, kypsymiseen ja aistittaviin ominaisuuksiin sekä juustoheran saantoon ja koostumukseen. Natiivien ja juustoherasta valmistettujen heraproteiinikonsentraattien toiminnallisia ominaisuuksia ja koostumuksia vertailtiin. Lisäksi vertailtiin juustomaidon esikäsittelymenetelmien, korkeapastöroinnin (HH), mikrosuodatuksen (MF) ja ultrasuodatuksen (UF), vaikutusta herasta valmistettujen heraproteiinikonsentraattien koostumukseen.

Tutkituilla polymeerisillä *spiral wound* -mikrosuodatuskalvoilla havaittiin 38.5% alhaisempi energiankulutus, 30.1% suurempi heraproteiinien pidättyminen retentaattiin ja 81.9% alhaisempi permeaattivirtaus verrattuna keraamisiin suodatuskalvoihin. Kaikki tutkitut mikrosuodatuskalvot olivat soveltuvia pääheraproteiinien erottamiseen maidosta. Optimaalisen emmental-juustomaidon laktoosipitoisuuden todettiin olevan yli 3.2%. Heraproteiinien ja tuhkapitoisuuden alentaminen juustomaidossa suurilla konsentrointikertoimilla (CF) tehosti juuston kypsymistä. Heraproteiinipitoisuuden alentaminen juustomaidossa lisäsi kaseiinimakropeptidien (CMP) osuutta juustoheran proteiinista. Maidon heraproteiinin, laktoosin ja tuhkapitoisuuden alentaminen lyhensi maidon juoksettumisaikaa ja lisäsi juoksettuman kovuutta. Juustosaanto raakamaidosta laskettuna oli alhaisempi mikrosuodatetuilla maidoilla johtuen natiivin heran muodostumisesta.

Maidon pääheraproteiinien, α-laktalbumiinin (α-LA) ja β-laktoglobuliinin (β-LG), pitoisuudet olivat merkittävästi korkeammat vertailuherassa. Tämä osoitti, että maidon korkeapastörointi, mikrosuodatus ja ultrasuodatus maidon esikäsittelymenetelminä alensivat heraproteiinien määrää juustoherassa. Jopa alhaisilla konsentrointikertoimilla (CF 1.4) mikrosuodatus heikensi juustoheran ravitsemuksellista arvoa. Maidon tehokkaan hyödyntämisen kannalta tulisi pyrkiä mahdollisimman korkeaan natiivin heran määrään ja juustoheran kaseiinimakropeptidipitoisuuteen. Natiiveilla heraproteiinikonsentraateilla oli erinomaiset toiminnalliset ominaisuudet verrattuna juustoherasta valmistettuihin heraproteiinikonsentraatteihin. Natiivin heraproteiinikonsentraatin ja juustoherasta valmistetun heraproteiinikonsentraatin aminohappokoostumusten välillä havaittiin merkittäviä eroavaisuuksia.

PREFACE

The research described in this dissertation was carried out at Valio Research and Development (R&D), Process Technology, Helsinki; Special product factory, Valio Ltd, Lapinlahti; University of Helsinki, Dairy technology, Helsinki during the years 2004-2008.

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Helsinki, December 2009
Antti Heino

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles, referred to in the text by the Roman numerals I-VI.

I. Heino, A., Uusi-Rauva, J., and Outinen, M. 2008. Microfiltration of milk I: Cheese milk modification by micro- and ultrafiltration and the effect of Emmental cheese quality. Milk Science International, 63, 279-282.

II. Outinen, M., Heino, A., and Uusi-Rauva, J. 2008. Microfiltration of milk II: Influence of the concentration factor on the composition of Emmental cheese milk and the κ-casein macropeptide content of the whey. Milk Science International, 63, 305-308.

III. Heino, A., Uusi-Rauva, J., and Outinen, M. 2009. Microfiltration of milk III: Impact of milk modification on milk coagulation kinetics. Milk Science International, 64, 128-131.

IV. Heino, A., Uusi-Rauva, J., and Outinen, M. 2010. Pretreatment methods of Edam cheese milk. Effect on cheese yield and quality. LWT-Food Science and Technology, doi:10.1016/j.lwt.2009.11.004

V. Outinen, M., Heino, A., and Uusi-Rauva, J. Pretreatment methods of Edam cheese milk. Effect on the whey composition. Manuscript (accepted for publication at LWT-Food Science and Technology).

VI. Heino, A., Uusi-Rauva, J., Rantamäki, P., and Tossavainen, O. 2007. Functional properties of native and cheese whey protein concentrate powders. International Journal of Dairy Technology, 60, 277-285.

RESEARCH INPUT AND AUTHORSHIP OF ARTICLES I-VI

I: Antti Heino planned and performed the tests, processed the results and wrote the article together with Janne Uusi-Rauva and Marko Outinen. Test trials were performed with Jyri Rekonen and Janne Uusi-Rauva.

II: Antti Heino planned the test trials and processed the results with Marko Outinen. Antti Heino wrote the article together with Marko Outinen and Janne Uusi-Rauva.

III: Antti Heino planned the test trials and processed the results with Janne Uusi-Rauva. Antti Heino wrote the article together with Janne Uusi-Rauva and Marko Outinen.

IV: Antti Heino planned the test trials, performed the tests, processed the results and wrote the article together with Janne Uusi-Rauva and Marko Outinen.

V: Antti Heino planned the test trials, performed the tests, processed the results and wrote the manuscript together with Marko Outinen and Janne Uusi-Rauva.

VI: Antti Heino planned the tests, made the test powders, processed the results and wrote the article together with Janne Uusi-Rauva and Pirjo Rantamäki. Except the viscosity, functional properties of powders were studied by Pirjo Rantamäki and Seija Tuomarmäki.

OTHER RELEVANT PUBLICATIONS

Heino, A., Outinen, M., and Uusi-Rauva, J. Removal of whey proteins from skimmed milk with polymeric microfiltration membranes. Manuscript (accepted for publication at Milk Science International).

Outinen, M., Heino, A., and Uusi-Rauva, J. 2010. Polymeric microfiltration of skimmed milk in Edam cheese process I. Effect of the concentration factor on the composition of vat milk and whey. Milk Science International, 65, 6-10.

Outinen, M., Heino, A., and Uusi-Rauva, J. Polymeric microfiltration of skimmed milk in Edam cheese process II. Effect on the composition and nutritional quality of whey protein concentrate. Manuscript (accepted for publication at Milk Science International).

Outinen, M., Rantamäki, P., and Heino, A. 2009. Effect of milk pretreatment on the whey composition and whey powder functionality. Journal of Food Science, doi: 10.1111/j.1750-3841.2009.01382.x

LIST OF ABBREVIATIONS

ρ	density (kg/m^3)
μ	dynamic viscosity of feed (Pa s)
δ	layer near a membrane surface where tangential flow is not turbulent (m)
ν	linear flow (m/s)
η	viscosity (Pa s)
μ_p	dynamic viscosity of permeate (Pa s)
ΔP_l	pressure drop over membrane (bar)
μ_r	dynamic viscosity of retentate (Pa s)
ΔV	permeate volume (L)
τ_w	wall shear stress (N/m^2)
A	membrane surface area (m^2)
a	particle radius (μm)
A40	curd firmness 40 min after rennet addition (mm)
ACY_r	adjusted cheese yield calculated from raw milk (%)
ACY_v	adjusted cheese yield calculated from vat milk (%)
Ala	alanine
BSA	bovine serum albumin
$CaCl_2$	calcium chloride
C_b	retentate concentration (g/100mL)
CF	concentration factor
cfu	colony forming unit
ngCMP	non-glycosylated caseinomacropeptide
CMP	caseinomacropeptides (ngCMP+GMP)
CN	casein nitrogen
CR	vat milk component recovery (%)
$CuSO_4$	copper sulfate
$C_{\beta\text{-LA, permeate}}$	concentration of β-LG in permeate (g/100 mL)
$C_{\beta\text{-LG, retentate}}$	concentration of β-LG in retentate (g/100 mL)
$C_{WP, permeate}$	concentration of whey proteins in permeate (g/100 mL)
$C_{WP, retentate}$	concentration of whey proteins in retentate (g/100 mL)
CWPC	cheese whey protein concentrate
CY_r	cheese yield calculated from raw milk (%)
CY_v	cheese yield calculated from vat milk (%)
$C_{\alpha\text{-LA}}$	concentration of α-LA
$C_{\beta\text{-LG}}$	concentration of β-LG
d	hydraulic diameter of filtration channel (m)
Da	molecular size, Dalton
E	energy consumption (kW)
EC	emulsifying capacity (g/mg)
FD	freeze drying
FDB	fat on a dry basis (g/kg)
GMP	glycosylated caseinomacropeptide
GP	gradient permeability membrane
HF	hollow fibre membrane
HH WPC	cheese whey protein concentrate made from high temperature heat treated milk
HH	high temperature heat treatment
Ig	immunoglobulin
IgG	immunoglobulin G
Ile	isoleucine
INRA	Institut National de la Recherche Agronomique

J	permeate flux (kg/m^{2}s or L/m^{2}h)
K20	time when a curd firmness of 20 mm was achieved (min)
K20-RCT	curd firmness of 20 mm (time) corrected for rennet clotting time (min)
L	length of membrane channel (m)
Leu	leucine
LF	lactoferrin
Lys	lysine
MF WPC	cheese whey protein concentrate made from microfiltrated milk
MF	microfiltration
MNFS	moisture of the non fat substance (g/kg)
M_{WP}	mass flux of whey proteins (kg/m^{2}h)
N	compression force, newton
NF	nanofiltration
NPN	non-protein nitrogen
NPN-P	non-protein nitrogen converted to protein equivalent by multiplying by 6.38
NWP	native whey protein
NWPC	native whey protein concentrate
NWPI	native whey protein isolate
PES	polyethersulfone
PKU	phenylketonuria
Pro	proline
PVDF	polymeric polyvinylidene fluoride
$P_{\beta\text{-LG}}$	permeation of β-lactoglobulin
P_{WP}	permeation of whey proteins
$Q_{\alpha\text{-LA}}$	relative amount of α-LA
$Q_{\beta\text{-LG}}$	relative amount of β-LG
R	overall filtration resistance (m^{2}kg^{-1})
R_c	filter cake resistance (m^{2}kg^{-1})
RCT	rennet clotting time (min)
Re	Reynolds number
REF WPC	cheese whey protein concentrate made from reference milk
R_{ir}	sum of an irreversible fouling (m^{2}kg^{-1})
R_m	intrinsic membrane resistance (m^{2}kg^{-1})
RO	reverse osmosis
RP-HPLC	reverse phase high pressure liquid chromatography
R_r	reversible fouling and polarisation effect (m^{2}kg^{-1})
RY	recovery yield of milk component in native or cheese whey (%)
SD	spray drying
SDS-PAGE	sodium docecyl sulphate polyacrylamide gel electrophoresis
SH-	disulphide bond
SW	spiral wound membrane element
T	temperature (°C)
t	time (s)
TFA	titratable fatty acid
Thr	threonine
TMP	transmembrane pressure (bar)
TN	total nitrogen
TN	total nitrogen (g/100g)
TP	total protein
Trp	tryptophan
TS	total solids (% or g/100g)
UF WPC	cheese whey protein concentrate made from ultrafiltrated milk

UF	ultrafiltration
UHT	ultra high heat treatment
UTP	uniform transmembrane pressure (bar)
UV	ultraviolet light
WP	whey protein
WPC	whey protein concentrate
WPC35	35 % whey protein concentrate
WPN	whey protein nitrogen
α-LA	α-lactalbumin
β-LG	β-lactoglobulin

LIST OF FIGURES

LIST OF TABLES

1 INTRODUCTION

Membrane filtration has been used in milk processing for several decades and nowadays it is one of the most important processing techniques in the dairy industry. Membrane filtration is widely used in milk and whey concentration and in producing process water from flush water. The main applications in dairy processes are milk concentration by ultrafiltration (UF), cheese or milk permeate concentration by nanofiltration (NF) or reverse osmosis (RO) as well as process water manufacture by RO. Microfiltration (MF) is a membrane filtration process in which tangential flow is used to sustain stable permeate flux in a porosity range of 0.05-10 μm. Typically in dairy processes, microfiltration has been used for starter concentration, cheese brine water clarification or defatting of cheese whey. Membrane filtration differs from other basic processes due to membrane characteristics. Microfiltration membranes are very often made of ceramics, which prolongs membrane lifetime and facilitates disinfection with steam or chemicals. Traditional polymeric membrane filters can also have a long lifetime when the membranes are used in suitable conditions with the recommended parameters.

Milk microfiltration for separating casein micelles from serum whey proteins was described already over twenty years ago (Maubois et al., 1987). Separation of whey proteins from milk and native whey protein reduction in milk microfiltrate was presented by Kulozik and Kersten (2002). Typically whey proteins are separated from milk using 0.05-0.2 μm ceramic membranes with low transmembrane pressure (TMP) values (0.1 to 1.0 bar) and high tangential flow rates (3 to 8 ms^{-1}) (Gésan-Guiziou et al., 1999b). In some previous studies whey proteins from skimmed milk were separated using polymeric microfiltration membranes, with satisfactory permeate flux and whey protein permeation (Govindasamy-Lucey et al., 2007; Lawrence et al., 2008). Permeation of whey proteins and permeate flux values together describe the mass flux of whey proteins, which plays the main role in whey protein separation processes (Piry et al., 2008). This mass flux of whey proteins can be converted into processing costs, which can be calculated as costs of whey protein mass flux per kilogram of protein.

Milk and milk-based liquids are difficult to filter due to protein fouling of membranes and precipitation of minerals. Membrane fouling in food applications causes a need for efficient cleaning to secure hygienic production and to restore membrane performance (Gésan et al., 1995b).

Separation of milk components is mainly affected by membrane pore size homogeneity, concentration polarization phenomena and membrane fouling (Jimenez-Lopez et al., 2008). There has been considerable progress during recent years in microfiltration using new types of membranes for casein separation from whey proteins. Whey protein separation was earlier possible only with ceramic membranes due to the requirement for narrow membrane pore size distribution (Zulewska et al., 2009).

Traditionally, cheese milk pretreatment alternatives have been ultrafiltration and high temperature heat treatment. These methods have been used for increasing milk component recovery in cheese (Guinee et al., 1995; Guinee et al., 2006). In all cases a large amount of whey is released, the quality of which depends on the cheese process.

By using microfiltration as a cheese milk pretreatment method it is possible to standardize cheese milk protein, lactose and ash compositions. This means separation of whey proteins and some of the lactose and minerals before milk coagulation. In this way it is possible to create ideal cheese milk, in which the necessary milk components for the cheese manufacturing process are present in suitable concentrations. Milk components which are removed before the cheese manufacturing process can be further processed to new types of products without any cheese components.

Milk pretreatment methods such as microfiltration in cheese manufacture have impacts on cheese yield, texture and sensory quality as well as on milk coagulation properties. In addition, modification of cheese milk affects the cheese whey amount, quality and usability as well as the functional properties of whey products.

The impact of cheese milk modification was studied by using different microfiltration processes. Cheese milk component recovery was evaluated in cheese and whey. Modified milk coagulation and the effects of milk minerals, lactose and whey protein concentrations were studied. Microfiltration as a cheese milk protein standardization method was compared to ultrafiltration and high temperature heat treatment methods.

2 LITERATURE REVIEW

2.1 The composition of milk and milk fractionation

Bovine milk has been very important part of human nutrition thousands of years. Milk has been used for human nutrition when it contains many essential components for human nutrition as well as it is good source of energy. The fractionation technology like membrane filtration is new way to utilize milk components for human nutrition in best possible way.

2.1.1 The composition of milk

Bovine milk consists of water (86-88%), fats (3-5%), proteins (3.3-3.6%), lactose (4.5-5.0%), salts (0.7%) and enzymes as well as many other minor components (Jenness and Patton, 1959). For this study milk proteins were the most important milk components. Milk proteins are divided to caseins and whey proteins. The main whey proteins β-lactoglobulin (β-LG), α-lactalbumin (α-LA) and bovine serum albumin (BSA) are 20% of total milk proteins (w/w). Caseins are the main milk proteins and in bovine milk these are on micellar form. Casein micelles are formed of individual submicelles α_{s1}-, α_{s2}-, β-, κ- and γ-caseins with calcium phosphate ($Ca_6(PO_4)_6$) clusters (Figure 1).

2.1.1.1 Casein micelles and whey proteins

The structure of casein micelles, denaturation of whey proteins and interaction of denatured whey proteins with casein micelles are presented on Figure 1. Whey proteins are hydrophilic and they are separated during milk coagulation (Kammerlehner, 1986). They are considerably smaller (15-130 kDa) than casein submicelles (500 kDa) but they are sensitive to heat (Andrews, 1964; Fox, 2001). Caseins are phosphorylated molecules and they have no secondary, tertiary or quaternary structures. Whey proteins are not phosphorylated but they are globular proteins with secondary, tertiary and quaternary structures which are stabilized with intramolecular disulphide (SH-) bonds (Fox, 2001). Casein micelles are heat stable but denatured whey proteins are attached to casein micelles with covalent bonds (Tran Le et al., 2008). Therefore whey proteins have a negative influence on casein micelle coagulation because chymosin enzyme has fewer open sites to remove hydrophilic caseinomacropeptide (CMP, the hydrophilic part of κ-casein) from the micelle surface. CMP formation is the main

phenomenon in milk coagulation (Bönisch et al., 2008) and in cheese manufacture (Kammerlehner, 1986).

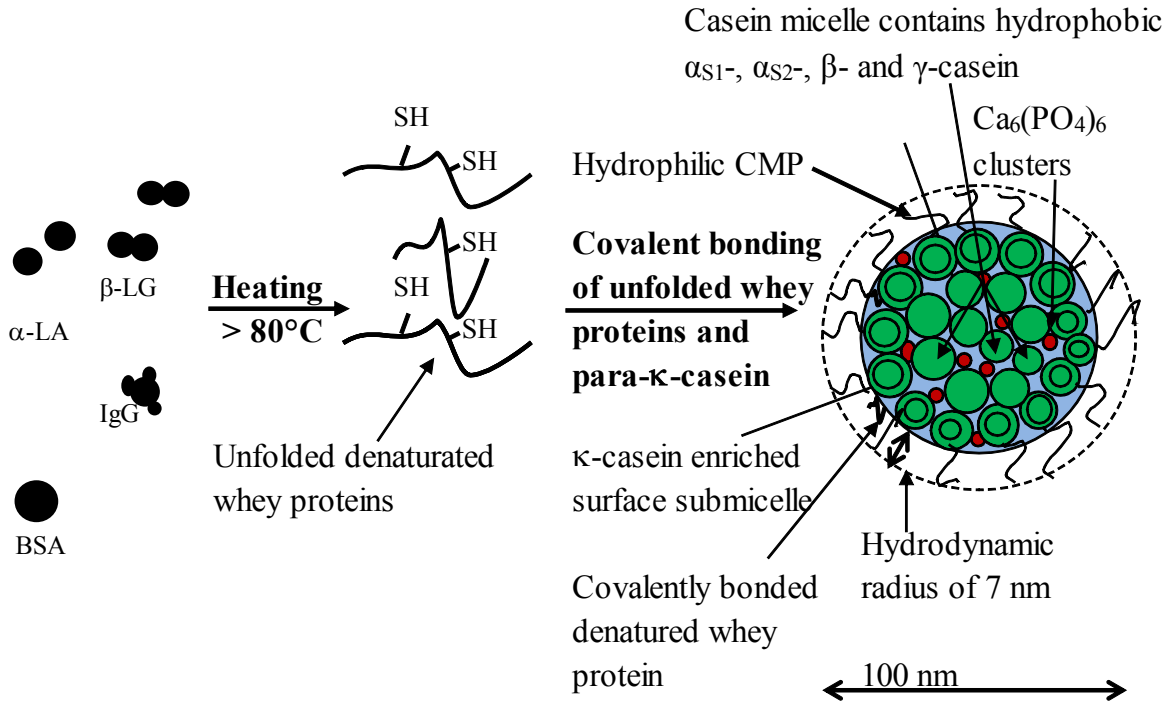

Figure 1. Casein micelle structure, whey proteins and attachment of whey proteins to casein micelles. CMP=caseinomacropeptide, $Ca_6(PO_4)_6$ = calcium phosphate cluster, SH=disulphide bond which has been opened during heating.

2.1.2 Fractionation of milk by membrane filtration

Milk can be fractionated to many different fractions by using membrane filtration techniques. These pressure-driven filtration techniques are microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO). RO technology only concentrates milk, whereas the other techniques can be used for milk fractionation. A filtration spectrum of membrane filtration techniques, main milk components and membrane porosity values is presented in Figure 2.

In this study microfiltration (0.1 μm and 800 kDa) and ultrafiltration (10 kDa) were used; the differences between these techniques were in the separation of whey proteins from milk. Microfiltration concentrated casein micelles but passed whey proteins through the membrane, whereas ultrafiltration concentrated both casein micelles and whey proteins.

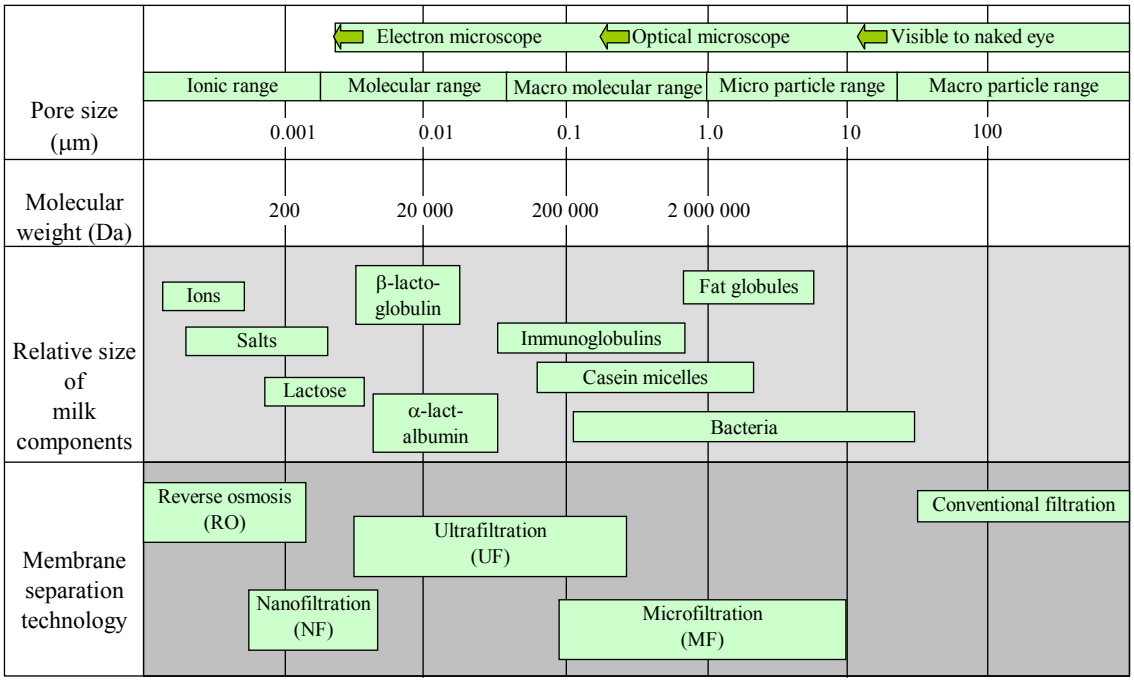

Figure 2. Membrane filtration techniques for milk fractionation, main milk components, molecular and particle sizes of milk components. (Adapted from Jensen and Køningsfeldt, 2000)

2.2 Microfiltration

Microfiltration (MF) technology is one type of pressure driven membrane filtration, which is used for separating particles, microbes or molecules from liquids. MF technology is widely used in the pharmaceutical, chemical, mining and food industries. MF as a filtration technology is between ultrafiltration (UF) and coarse filtration. MF is the oldest technique among filtration techniques and the first cellulose MF membranes were designed almost one hundred years ago (Ripperger and Altmann, 2002). The importance of tangential flow in MF filtration was realized in 1907. Tangential flow influences cake layer formation and the increase of filtration pressure during filtration (Bechhold, 1907). It is important to sustain stable permeate flow rate during continuous operation and therefore cake layer formation should be minimized. MF employs membranes with a mean pore size of 0.02-10 μm. Modern microfiltration membranes are made to a particular pore size and therefore by using a combination of membranes different fractions can be obtained from a single feed liquid.

2.3 Milk microfiltration techniques

2.3.1 Separation of micellar casein from milk

When micellar casein is concentrated by microfiltration (pore size 0.05-0.2 μm) it is very important to reduce membrane fouling. Fouling reduces whey protein permeation, which is the main feature of micellar casein concentration. In all cases small amounts of whey proteins are found from the micellar casein fraction after filtration (Brans et al., 2004). The concentration of micellar casein can be performed from full fat milk or skimmed milk, and it can account for up to 95% of total protein. This kind of high-casein retentate can be obtained with a microfiltration process, which includes a diafiltration step. When micellar casein micelles are concentrated the milk microfiltrate permeate contains milk whey proteins, and is called native or ideal whey. The native whey is a microbiologically sterile and clear permeate, the composition of which is close to that of sweet cheese whey (Fauquant et al., 1988). This kind of permeate does not contain caseinomacropeptides, cheese starters or chymosin. In addition, the native whey does not contain fat, bacteriophages or partially denaturated whey proteins. Traditionally, casein concentration has been performed with ceramic microfiltration membranes by using the uniform transmembrane pressure (UTP) principle with high tangential flow velocity (>6 m/s) and 50-55°C filtration temperature (Maubois, 2002).

Native whey is formed when micellar casein concentrate is manufactured. Native whey contains native whey proteins, which have excellent functional properties, and therefore the technological and economical value of the native whey is higher than that of the standard sweet cheese whey (Maubois, 2002). Native whey has the same pH as milk, unlike cheese whey which is always more acidic (Maubois et al., 2001). If the native whey is further concentrated by ultrafiltration, native whey protein concentrate (NWPC) or isolate (NWPI) is formed (Maubois et al., 2001). NWPC can be dried and used in applications in which excellent solubility, foaming and gel forming properties are required (Østergaard, 2003). Native whey protein has foaming properties equal to those of egg white (Punidadas and Rizvi, 1998).

The nutritional value of native whey protein is higher than that of cheese whey due to its different amino acid composition. This difference has led to increased interest in native whey protein utilization. Utilization of the native whey proteins in human nutrition (Boirie et al.,

1997), especially as a raw material for weight balancing products (Burton-Freeman, 2008) or baby food products, is based on the lack of the glycosylated part of the caseinomacropeptide or in other word glycomacropeptide (GMP) molecule (Rigo et al., 2001). Lack of GMP is an important feature in baby food products because GMP contains 20% more threonine (Thr) than human milk (Boehm et al., 1998). GMP is thought to cause hyperthreoninemia in preterm infants and therefore its content in baby food products should be low. It is possible to separate individual whey proteins (β-lactoglobulin, α-lactalbumin, osteopontin) from native whey by chromatographic processes (Maubois et al., 2001). Individual whey proteins and hydrolyzed whey proteins can have various biological activities. For example, β-lactoglobulin (β-LG) anticarcinogenic tripeptide may protect against intestinal cancer (McIntosh et al., 1995).

Micellar casein fraction can be used in cheese manufacture to replace milk, or for cheese milk protein standardization. From micellar casein it is possible to manufacture pure β-casein or caseinomacropeptides (CMP) by further processing (Maubois and Ollivier, 1997).

2.3.2 Microfiltration membranes in whey protein separation

Whey protein separation with microfiltration (pore size 0.05-0.2 μm) can be performed in batch or continuous mode. In industrial processes continuous filtration is used due to easier control and longer filtration times. In milk microfiltration ceramic membranes, which can tolerate high temperatures and both low and high pH values, have traditionally been used. Milk microfiltration with ceramic membranes is traditionally performed at 50-55°C with transmembrane pressure (TMP) 0.1-1.0 bar and with high tangential flow rates (>6 m/s) (Sachdeva and Buchheim, 1997). Tubular ceramic membranes are able to give a permeate flux rate of 55-65 L/m^2h (mean concentration factor from 1 to 4) with these parameters. Lower filtration temperatures and higher TMP values lead to lower permeate flux and higher whey protein retention. In industrial milk microfiltration, ceramic membranes (0.05-0.2 μm) need high tangential flow in order to reach high permeate fluxes (Maubois et al., 1987).

Polymeric microfiltration systems are not widely used in milk concentration or fractionation due to their high fouling rate and lack of data on their properties and applicability. According to Saboya and Maubois (2000), polymeric membranes cannot be used for on milk fractionation because of poor retention of caseins and low permeate flux. In addition,

polymeric membranes have poor mechanical, chemical and heat stability. However, some very recent studies have shown that the new generation of polymeric microfiltration membranes can be used for milk fractionation (Govindasamy-Lucey et al., 2007; Lawrence et al., 2008). In fact, polymeric microfiltration membranes can be used at low temperatures (5-10ºC), which is not reasonable for high energy consuming ceramic systems. Lawrence et al. (2008) filtered milk at 10ºC with 1.5 bar TMP and 0.4 m/s tangential flow rate. Permeate flux with 0.3 μm membranes varied from 6 to 18 L/m^2h with 98% casein and 69% β-lactoglobulin retention. Important factors for industrial applications are the investment and running costs of membrane systems, in which polymeric systems have a clear advantage (Schier, 2007). However, the lifetime of polymeric membranes is much lower than that of ceramic membranes and they are less tolerant to cleaning chemicals, possibly causing higher variation in retention values during the membrane lifetime (Schier, 2007).

It is clear that membrane pore size should vary as little as possible in milk microfiltration because milk caseins and whey proteins have only a rather small difference in molecular mass. Pore size distribution in polymeric membranes has been too wide for whey protein separation from milk (Brans et al., 2004). If the pore size distribution is too wide, larger particles such as casein micelles pass through larger pores while at the same time smaller particles pass through smaller pores. Fouling reduces the amount of open pores and influences permeate flux and membrane selectivity. If larger pores are blocked first, retention increases and permeate flux decreases sharply (Brans et al., 2004).

2.4 Microfiltration equipment

All milk microfiltration systems apply tangential flow or shear induced vibration, which generates turbulent flow near the membrane surface. Tangential flow causes pressure drop over the membrane channel and this pressure drop means higher pressure at the input compared to the output. In milk microfiltration this pressure drop (tangential flow rate) can be higher than the optimal mean transmembrane pressure (TMP). High tangential flow rate is needed to reduce cake layer thickness and compactness on the membrane surface. The cake layer forms a secondary layer on the membrane surface and the quality of this secondary layer affects the permeate flux and retention values (Gésan-Guiziou et al., 2000). In ceramic systems a pressure drop has also been generated on the permeate side in order to reduce TMP difference in different areas of the membrane surface (Kessler, 1997). This idea has been called the uniform transmembrane pressure (UTP) principle (Sandblom, 1978). One

disadvantage in the use of the UTP principle has been higher energy consumption, because the permeate side also needs tangential flow (Figure 3, B). An advantage of the UTP principle is lower membrane fouling and only minor changes in permeability and permeate flux during filtration (Saboya and Maubois, 2000). To reduce energy consumption in UTP principle filtration, membrane manufacturers have developed gradient porosity membranes (Pall Exekia, GP®-membranes), in which a pressure gradient has been generated in the membrane surface or support layer. Gradient membranes are made for specific applications and process conditions and for this reason they are less flexible than UTP-membranes, with which the pressure gradient can be adjusted to follow product viscosity changes. Polymeric membranes (Figure 3, A) use non-UTP mode, because at present organic spiral wound (SW) or hollow fiber (HF) membranes are not designed to tolerate permeate circulation. Therefore filtration performance of polymeric membranes varies widely along the length of the membrane.

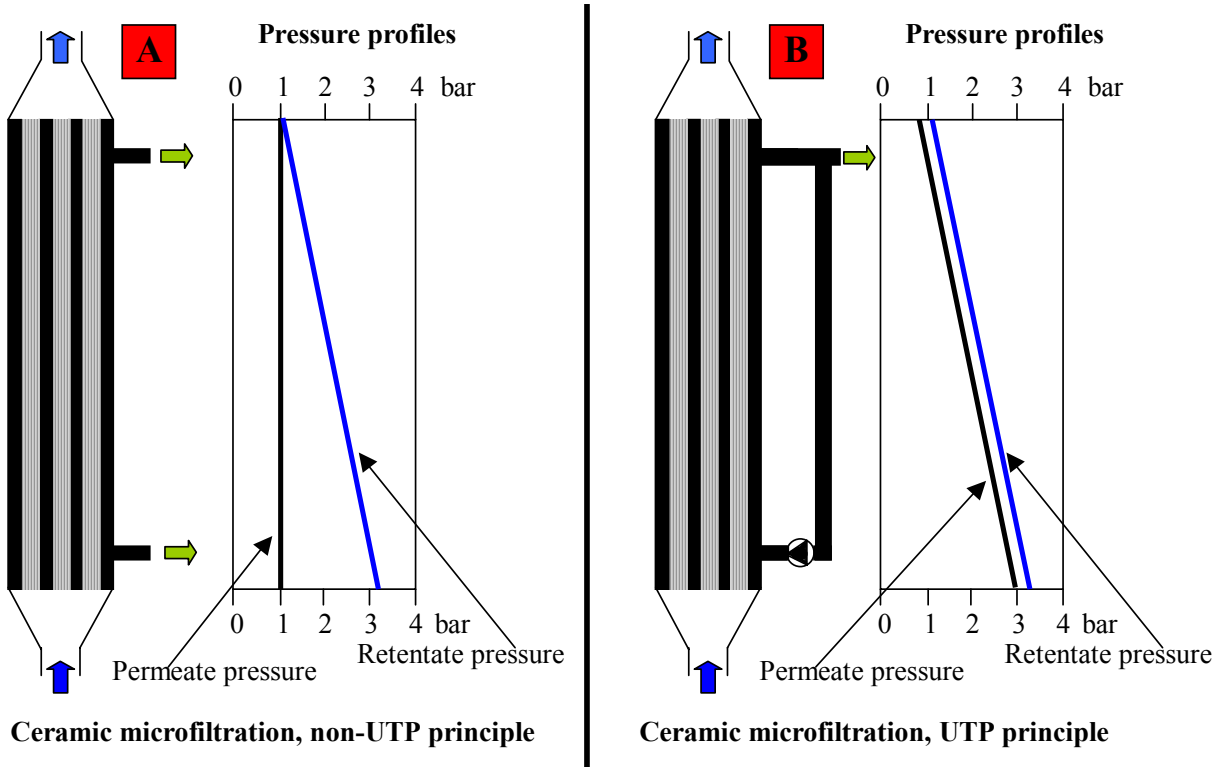

Figure 3. Pressure profiles in (A) non-uniform transmembrane pressure (non-UTP) and (B) uniform transmembrane pressure (UTP) principle ceramic microfiltration systems (Kessler, 1997).

2.4.1 New types of microfiltration membranes

There has recently been an interest in the development of membranes with lower resistance to permeate formation, improved selectivity, narrow pore size distribution and lower tendency to fouling as well as lower energy consumption. Microsieves have also been developed to meet these needs, but they are not yet widely used. Metal microfilters have been made for the 2-10 μm pore size range and these have had high permeate flux rates and low tendency to fouling (Holdich et al., 2003). Track etched membranes also have narrow pore size variation due to a very precise manufacturing process (Apel, 2001). Inorganic silicon microsieves have extremely low membrane resistance and exact pore size distribution and pore shape (Kuiper et al., 1998). Polymeric microfiltration membranes are traditionally hydrophilic, and modification of membrane surfaces to be more hydrophobic can reduce membrane and protein interactions and protein adsorption. Several studies have also been made with polymeric membranes which have been coated, chemically modified or polymerized to reduce membrane fouling (Pieracci et al., 2000; Chen and Belfort, 1999; Blanco et al., 2006).

2.5 Principles of microfiltration

2.5.1 Membrane fouling

Fouling can be divided into reversible and irreversible fouling. Reversible fouling can be removed during or after filtration with a water flush. Irreversible fouling is more difficult to remove from the membrane surface or pores by cleaning treatments (Gésan-Guiziou et al., 1999a). Membrane fouling is dependent on permeate flux. During filtration the membrane retains large particles which are not able to go through membrane, and these particles form a cake layer. This cake layer reduces permeate flux and therefore increases membrane resistance (R_c). Permeate flux (J) and retentate concentration (C_b) define the resistance of the concentration polarization layer (Gésan et al., 1995a). Movement of retained particles towards the membrane surface is related to permeate flux and retentate concentration. On the membrane surface particles move with a laminar flow. Combination of Brownian motion and wall shear stress forces, have greatest influence on those particles which are smaller than 100 nm. The mean diameter of milk casein micelles is about 100 to 220 nm (de Kruif and Holt, 2003; Udabage et al., 2003), which means than Brownian motion has a greater impact on casein movement that shear stress forces. If the Reynolds number value is higher than 1500,

shear stress forces will have a major impact. Milk casein concentration influences the Re value: with higher concentrations or higher viscosity values, higher tangential flow must be used to reach the same Re value. Increase in concentration also increases the thickness and density of the cake layer, which affects particle movement on the membrane surface (Zeman and Zydney, 1996). Slow movement of the cake layer reduces particle attachment to the membrane surface. The wall shear stress (τ_w) is the force which removes particles from the membrane surface and it can be determined by using equation 1, where L is the length of the membrane, d is the height of the membrane channel and ΔP_l is the pressure drop over the membrane (Gésan-Guiziou et al., 2000).

$$\tau_w = \frac{d \Delta P_l}{4 L} \qquad (1)$$

Permeate flux is mainly the result of τ_w value and particle size and concentration (Vadi and Rizvi, 2001). The permeate flux (J), which is the main factor in membrane filtration, can be determined by equation 2. This equation is based on Darcy's law, in which μ is the dynamic viscosity, R is overall hydraulic resistance (the sum of membrane, fouling and cake layer resistances), and ΔP_l is the pressure drop over the membrane.

$$J = \frac{\Delta P_l}{\mu R} \qquad (2)$$

The membrane itself, fouling and the cake layer cause resistance to permeate flux. Membrane resistance (R_m) is dependent on membrane thickness, membrane support layer thickness, mean pore size and liquid route through the membrane. Cake layer resistance (R_c) is higher with higher filtration pressures and lower with higher R_m values. R_c can be calculated with equation 3, in which μ_p is the permeate dynamic viscosity and ΔP_{TM} is the transmembrane pressure of filtration (Vadi and Rizvi, 2001).

$$R_c = \frac{\Delta P_{TM}}{\mu_p J} - R_m \qquad (3)$$

In continuous filtration, stable permeate flux is reached when overall filtration resistance (R) does not increase. This holds true if fouling is not detectable and the cake layer is not compressed. The rate of cake layer formation and disintegration is equal. However, at the very

beginning of filtration the reduction in permeate flux is not due to membrane fouling. This is the moment when the cake layer (gel layer with casein) over the membrane is formed. Fouling and permeate flux reach a steady state situation when particle flow to the surface is at the same level as particle removal from the cake layer, i.e. these two opposite processes are in equilibrium (James et al., 2003). The steady state filtration is the result of permeability of the gel layer and if TMP is higher the gel layer is thicker and more compact, increasing the cake layer resistance (R_c) value (James et al., 2003).

If permeate flux exceeds a critical value there is an irreversible particle attachment to the membrane surface during the first seconds of filtration (Howell, 1995). Fouling means irreversible particle attachment to the membrane surface, which cannot be flushed away. Biological liquids contain denaturated or aggregated proteins, which have a tendency to cause membrane fouling (Zeman and Zydney, 1996; Makardij et al., 1999). The susceptibility of a membrane to fouling can be reduced by using higher τ_w and lower ΔP_{tm} values, which decrease the height of the cake layer to a certain limit (Aubert et al., 1993).

Near the membrane surface the tangential flow is conciderably slower than in the centre of the flow channel. In the centre of the flow channel the flow is turbulent but it is reduced to laminar flow closer to the membrane surface. The thickness of this laminar flow layer (δ) is important because the layer contains particles which are smaller in diameter than the laminar layer thickness. In milk MF filtration, casein micelles are retained in this laminar flow layer if the layer is thicker than the casein micelles themselves.

Membrane fouling increases the hydraulic resistance (R) to permeate flow and this induces unfavourable impacts for process efficiency. On the membrane surface, protein fouling can occur due to four different mechanisms depending on process parameters, membrane structure and behaviour of proteins near the membrane surface or in the cake layer (Figure 4). Pore narrowing is a result of protein adsorption to the membrane surface due to solute and membrane electrical properties, especially if the difference in zeta potential is close to zero (Martinez et al., 2000). Pore plugging is possible even if pore size is larger than the particles, due to the aggregate formation on the membrane surface or inside the membrane structure (Güell et al., 1999). Internal membrane fouling reduces permeate flux and selectivity of the membrane if the membrane has a complex structure (Saxena et al., 2009). Many theoretical pore blocking and cake filtration models have been developed, but none of them has explained the fouling phenomena completely (Ho and Zydney, 2000).

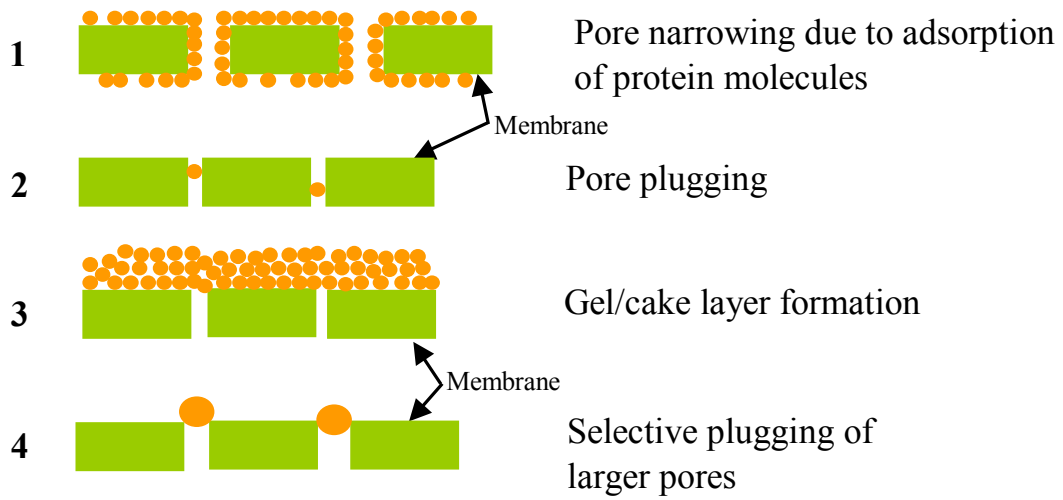

Figure 4. Mechanisms (1 to 4) causing membrane pore narrowing and plugging (Saxena et al., 2009).

2.5.2 Critical and limiting flux in microfiltration

Permeate flux (J) during filtration is usually defined as the system performance, which means the volume (ΔV) of permeate from a certain membrane area (A) in a given time (Δt). This is normally calculated using equation 4 (Makardij et al., 1999).

$$J = \frac{\Delta V / \Delta t}{A} \qquad (4)$$

During the past decade many studies in microfiltration have been carried out examining critical and limiting flux theory (Holdich et al., 2003; Huisman and Trägårdh, 1999; Gésan-Guiziou et al., 2000; Howell, 1995; Field et al., 1995). At least four different mechanisms cause erosion of the cake layer in microfiltration: inertial lift, wall shear stress diffusivity mechanism, Brownian diffusivity and liquid transport mechanism on the membrane surface (Belfort et al., 1994). By using these mechanisms it is possible to predict a critical flux (J_{crit}) from a microfiltration membrane. Critical flux is the maximum permeate flux value, which is obtained with linearly increasing transmembrane pressure at a value called critical transmembrane pressure (P_{crit}) (Figure 5). Below the critical flux value the permeate flux increases linearly with increasing TMP as in the case of water (hard form), or non-linearly (weak form) depending on liquid composition and viscosity (Metsämuuronen and Nyström, 2005). The following equation (5) describes the overall characteristics of permeate flux reduction (Field et al., 1995).

$$J = \frac{TMP}{\mu (R_m + R_{ir} + R_r)} \quad (5)$$

where R_{ir} is an irreversible fouling, μ feed viscocity, R_r reversible fouling and polarisation effect.

At values below the critical flux value selectivity of the membrane is better but at lower flux values the need for membrane area increases. Critical flux is dependent on wall shear stress, filtration temperature, characteristics of particles in liquid and membrane characteristic such as morphology and chemical membrane material (Makardij, 1999; Gironés et al., 2006). TMP values above the critical transmembrane pressure (P_{crit}) value increase permeate fluxes, but not linearly as below the critical permeate flux (J_{crit}) value. The limiting flux (J_{lim}) is the maximum permeate flux which can be obtained with limiting TMP (P_{lim}) value. TMP values above the limiting TMP value decrease permeate flux, as seen in Figure 5.

Filtration zones are presented in Figure 5, in which filtration zone I represents the zone of low permeate flux, low fouling rate and low retention of separated components. Milk filtration in zone II assumes high tangential flow rates to reduce membrane fouling. Increased tangential flow increases energy consumption and with certain tangential flow rates the optimal situation can be found in the region where permeate flux compared to energy consumption is highest (Gésan-Guiziou et al., 1999a). In zone III permeate flux and permeation of separated components are decreased, and therefore the filtration outside the optimal filtration range.

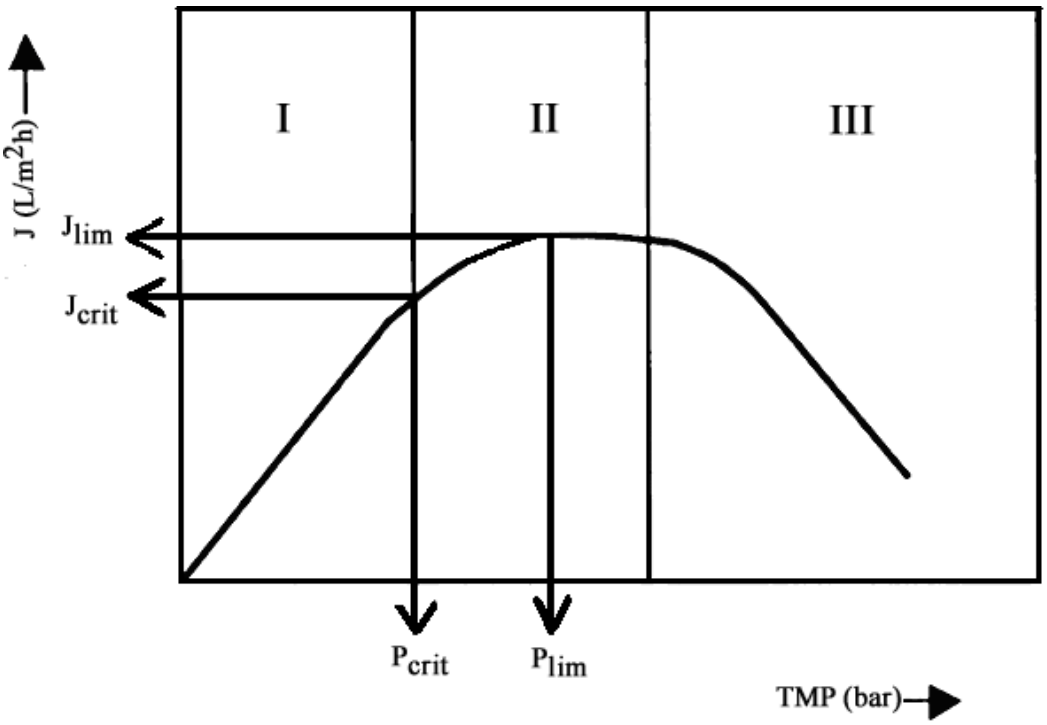

Figure 5. Effect of transmembrane pressure (TMP) in critical and limiting permeate flux (J) values and filtration zones I-III. P_{crit} = critical transmembrane pressure, P_{lim} = limiting transmembrane pressure, J_{crit} = critical permeate flux, J_{lim} = limiting permeate flux (Brans et al., 2004).

2.5.3 Membrane fouling in milk microfiltration

Hydrodynamical conditions such as tangential flow rate, wall shear stress and transmembrane pressure are the main factors affecting membrane performance during filtration. These factors affect membrane fouling and membrane performance and selectivity (Piry et al., 2008). Fouling in milk microfiltration starts with α-lactalbumin and β-lactoglobulin adsorption on the membrane surface during the first minutes of filtration. Bovine serum albumin (BSA) in milk also causes aggregates on top of the membrane surface, thus blocking the pores. Native or non-aggregated proteins are chemically attached to these whey protein aggregates by disulfide linkages (Kelly and Zydney, 1997). In addition, other fouling mechanisms caused by molecular interactions based on van der Waals forces, hydrophobic interactions, electrostatic interactions and hydrogen bonding exist (Marshall et al., 2003). Tong et al. (1988) reported that 95% of fouling layer proteins are whey proteins. Lee and Merson (1975) found that β-lactoglobulin has the highest potential for membrane fouling due to sheet-forming and because it constitutes 50% of whey proteins. BSA, immunoglobulins and β-lactoglobulin can induce anchor point formation for other proteins and cause thicker sheet formation (Lee and Merson, 1975). Milk minerals such as phosphorus and calcium also bind casein to whey proteins, and in this way formation of a thick fouling layer is possible (Vetier et al., 1988).

2.5.4 Reduction of fouling and increasing membrane performance

Many improvements in microfiltration technology have been reported in recent years. Improved membrane performance and reduced filtration costs can be attained using different methods (Table 1). However, many of these methods cannot be applied in large scale and some of them have a negative influence on filtered product quality (Brans et al., 2004).

Table 1. Methods and principles for improving ceramic membrane performance and disadvantages of these methods according to Brans et al. (2004).

Method	Principle	Disadvantage
High tangential flow with UTP principle	Improved erosion on membrane surface, wall shear stress, low TMP	High energy consumption, high investment and running costs
Turbulence promoters	Improve erosion effect on membrane surface	Difficult cleaning, increased energy consumption
Backpulsing	Removes cake layer with backpulse, higher pressure on permeate side (negative TMP)	Difficulties to control in large scale
Pulsated tangential flow	Creates velocity changes in the feed side	Difficult to control in large scale
Air slugs	Increases mixing and shearing on membrane surface	Difficult to control air slugs, causes foaming and denaturation of proteins
Scouring particles	Increases flow and wall shear stress on membrane surface	Wear of pumps and membranes, denaturation of proteins
Ultrasonic, acoustic waves	Removes attached particles by vibrations or cavitations	Increased energy consumption, denaturation of sensitive components
Vibrated membrane modules	Increases wall shear stress on membrane surface	Expensive equipment, up-scaling
Rotating membranes	Increases wall shear stress on membrane surface	Cleaning problems, up-scaling
Electric fields	Electric field removes charged particles from membrane surface	Electrolysis, energy consumption, gas production

Vibrated membrane modules could not be used in milk microfiltration for reasons of hygiene (Ding et al., 2002). Backpulse technology has an effect on the filtration performance and depending on backpulse interval, length and pressure profile (Guerra et al., 1997). Backpulses in large-scale equipment are damped and the effect is reduced more than in smaller filtration units (Jaffrin et al., 1994). Denaturation of proteins excludes ultrasonic methods in milk microfiltration (Villamiel and de Jong, 2000). Electric field has an effect on milk rancidity, and consequently has not been applied (Wakeman, 1998). New types of ceramic membranes, which have turbulence promoters, are reducing cake layer thickness. The turbulence promoters are able to increase permeate flux (J) and decrease energy consumption (Brossous et al., 2001).

2.6 Cheese manufacture

Cheeses have been made for over 5000 years, and it was recognized a long time ago that conversion of milk to cheese preserves valuable milk components in a compact way. Nowadays, numerous cheese varieties are made to fulfil human needs. Cheeses can be made from any milk source but economically the most important milk, due to its abundance, is bovine milk. Milk can be used for hard or soft cheese production, fresh or ripened cheese production or mould cheese production. Cheese manufacture starts with milk fat-to-protein standardization, including pasteurization or other heat treatment. Additives such as starters, calcium chloride ($CaCl_2$) and copper sulphate ($CuSO_4$) are added to cheese milk.

Calcium chloride is an important additive in cheese milk because free calcium accelerates milk coagulation, leads to a harder milk coagulum and increases cheese yield due to higher casein recovery in cheese (Fagan et al., 2007). $CaCl_2$ addition is very important with heat treated milks in which the free calcium content is otherwise reduced due to calcium phosphate formation, denaturation of whey proteins and attachment of denatured whey proteins to κ-casein. However, heat treated whey protein-free milk has not been observed to require $CaCl_2$ addition (Vasbinder et al., 2003). Another additive, copper sulphate ($CuSO_4$) is added traditionally only to Emmental cheese milk to improve carbon dioxide production and eye formation with *Propionibacterium* strains and to improve cheese proteolysis (Maurer et al., 1975). Cheese starters ferment milk lactose to lactic acid and reduce cheese pH to the desired level. Starters are mainly responsible for cheese flavour and for the whole complex ripening process (Kammerlehner, 1986). Chymosin enzyme is a necessary additive; without chymosin traditional cheese cannot be made (Kammerlehner, 1986).

After a short stirring step, chymosin is added and milk is coagulated for 30-45 min. Coagulated milk (curd) is cut and whey is traditionally partly removed from the cheese vat. Some water addition to curd can be made for diluting the lactose content of curd. After the water addition step, the curd is cooked and curd syneresis removes whey from the curd pieces. At the same time, starters are hydrolyzing the residual curd lactose to lactic acids. Finally, curd is moulded and pressed in the cheese moulds. After pressing, fresh cheeses are removed from the moulds, salted in a salt bath and ripened in different ways according to the cheese recipe (Kammerlehner, 1986).

In all cases milk casein is coagulated with rennet or with acid to separate milk serum and milk coagulum. For the cheese processor the main targets have always been good and stable cheese quality and high recovery of milk components in the cheese. Maximum recovery of the milk components has been attempted by high temperature heat treatment of cheese milk (Lawrence, 1993), concentration of milk by evaporation, concentration of milk proteins by ultra- or microfiltration (Guinee et al., 2006; Ur-Rehman et al., 2003), addition of denaturated and particulated whey proteins (Asher et al., 1992) or addition of calcium chloride ($CaCl_2$) (Wolfschoon-Pombo, 1997).

The traditional cheese process produces whey as a by-product, and usability of whey has become economically more important. Some cheese types produce acid whey, the usability of which is low due to its low pH value and therefore it has been important to reduce the amount of acid whey. Nowadays this is possible by microfiltration, in which native whey is removed before the cheese process (Schafroth et al., 2005). Production of native whey creates possibilities for better utilization of milk serum because cheese starters, rennet, lactic acid or cheese colour are not affected by the quality of whey (Maubois, 2002; Nelson and Barbano, 2005). Native whey proteins have a higher value than whey products or other milk products when protein functionality or nutritional value is important (Boirie et al., 1997). For the cheese processor the casein, salt and lactose content of milk have the greatest importance due to milk coagulation (Nelson and Barbano, 2005). Therefore the whey protein content of cheese milk is not important when whey proteins are passed to whey without milk high temperature heat treatment (Maubois et al., 2001). Separation technologies such as microfiltration are therefore excellent tools to maximize the economical value of milk.

2.6.1 Milk coagulation kinetics

Milk casein is precipitated when milk pH is lowered with acid near to about 4.6, or by using chymosin. Coagulation of casein micelles by action of chymosin enzyme is presented on Figure 6.

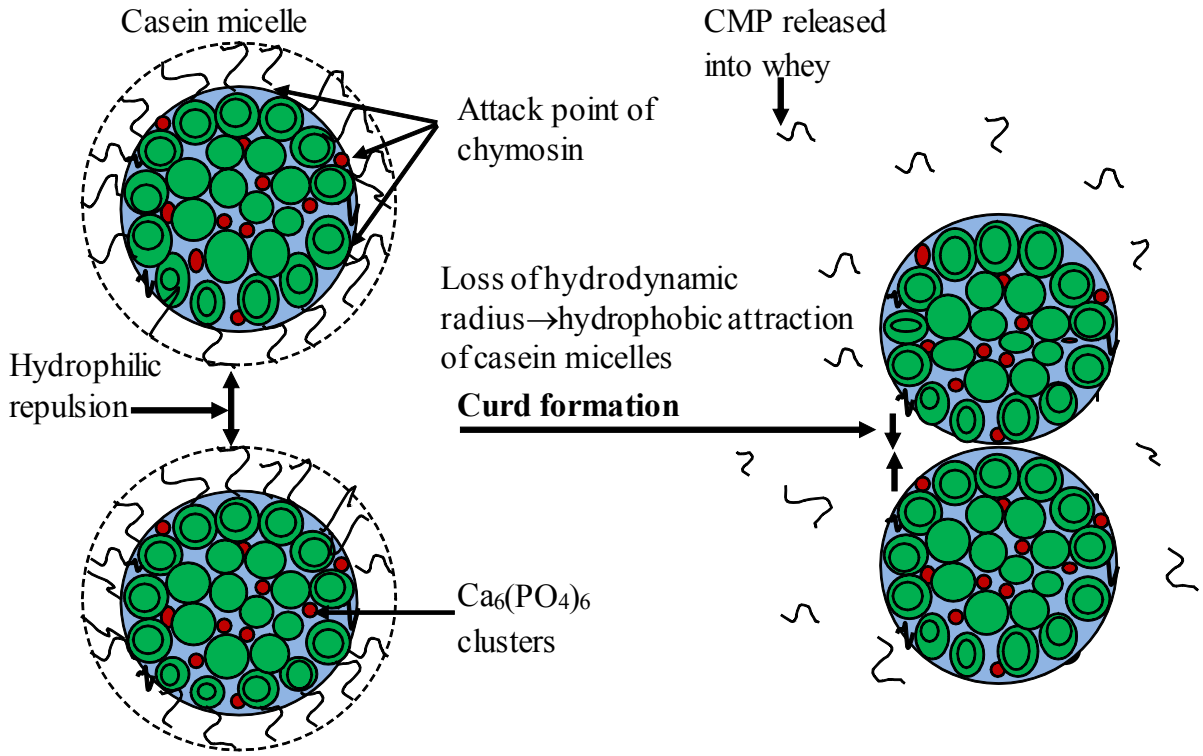

Figure 6. Coagulation of casein micelles by chymosin and cleavage of CMP.
CMP=caseinomacropeptide.

Milk coagulation is the first step in cheese manufacture. Rennet induces milk coagulation in three phases, i.e. enzymatic proteolysis, aggregation and gelification. Enzymatic proteolysis starts when chymosin contacts κ-casein and in this phase the caseinomacropeptide (CMP) is released into whey. The aggregation phase initiates when a sufficient amount of κ-casein is hydrolyzed. After that the repulsion forces between micelles are decreased and hydrophobic interactions are enhanced (Bönisch et al., 2008). The micelles form chains which require energy. This energy is obtained from casein particles. When CMP is released, electrical forces between micelles cause the formation of a casein matrix (Figure 6). When CMP is released from micelles the surface charge of CMP and micelles are opposite and therefore CMP can be removed with the aqueous phase. This supports the development of casein micelle matrix, which reduces or prevents Brownian motion. During gelification the distance between micelles is decreased and the structure of micelles is altered due to the stronger network and this causes whey syneresis from gel matrix (Kammerlehner, 1986). These phases can be understood as primary and secondary phases of the renneting process (Dalgleish, 1992). Milk coagulation is a critical phase in cheese manufacture, and has a drastic effect on the chemical and functional properties of cheese. Milk salt balance (Famelart et al., 1999; Lucey and Fox, 1993), pH (Famelart et al., 1996), quality (Ng-Kwai-Hang et al., 2002), and the amount (Steffl et al., 1999) of whey protein as well as the amount of rennet have an effect on milk

coagulation kinetics (McMahon and Brown, 1982). Caron et al. (1997) reported that higher protein levels in milk increase coagulation strength and reduce coagulation time, and that lower milk mineral contents result in a softer coagulum. Cutting time and properties of coagulation have significant effects on the cheese recovery yield, and consequently cheese processes should be adjusted in appropriate ways to optimize milk coagulation. Concentration of cheese milk casein by microfiltration (MF) is a method to reduce the amount of whey proteins in milk and to reduce cheese vat milk volume. MF of cheese milk reduces or even eliminates the release of cheese whey, if part or all of the whey has already been removed (as native whey) before the actual cheese process (Neocleous et al., 2002b).

The hydrophilic part of κ-casein f(106-169) which is released by the chymosin during the renneting of milk is about 12 to 14% of milk total casein. Of this amount ca. 50% is enzymatically cleaved into caseinomacropeptides (CMP) (Mollé and Léonil, 2005). This CMP can be further differentiated to non-glycosylated caseinomacropeptide (ngCMP) and glycomacropeptide (GMP) fractions. There is a considerable variation in the degree of glycosylation (Vreeman et al., 1986): the GMP to ngCMP ratio varies from 30:70 (Casal et al., 2005) to 60:40 (Lieske et al., 1996; Vreeman et al., 1986). The average caseinomacropeptide yield is 50 g/kg casein, consisting 20-25% of total proteins in rennet whey (Thomä-Worringer et al., 2006; Swaisgood, 2003; Vreeman et al., 1986). Glycomacropeptide is glycosylated and phosphorylated and the ngCMP is non-glycosylated but phosphorylated. GMP fraction contains sialic acids which is often terminal carbohydrate of glycans (Holland et al., 2006). This carbohydrate consists of five different glycans which can be varied due to genetic or posttranslational modifications. These CMP fractions can be separated with chromatographic methods (Tanimoto et al., 1992; Kreuß et al., 2009) and used for novel functional foods.

2.6.2 Microfiltration as a cheese milk pretreament method

Milk casein concentration by MF accelerates curd firmness and decreases coagulation time (Caron et al., 1997; Pierre et al., 1992; Maubois, 2002). It also reduces the need for additives, e.g. $CaCl_2$ (Schafroth et al., 2005), and enables stronger heat treatment for cheese milk (Samuelsson et al., 1997a). Microfiltration as a cheese milk pretreatment method affects the cheese process. When the milk protein level is standardized it is easier to minimize cheese quality changes and to optimize the cheese process (Klein and Lortal, 1999). In the cheese process, milk coagulation time can be reduced to half and a more rigid coagulum is obtained

when the coagulation rate is accelerated. Maximum strength of coagulum and therefore hardness of cheese measured using an (Instron Universal Testing Machine) in the compression test was increased from 50 newtons (N) to 70 N (40%) when MF was used as a milk pretreatment method (Neocleous et al., 2002a). Casein and fat recoveries to cheese are increased, reducing casein and fat contents in cheese whey. According to Daviau (2000a), microfiltration increases the cheese yield by 2-4% as a result of the increased level of milk protein.

2.6.3 Microfiltrated milk in fresh cheese manufacture

Fresh cheeses can be made from ultrafiltrated or microfiltrated milk when the milk proteins are concentrated up to 5-8 times. After the concentration step, starter and rennet are added to milk and after coagulation cheese is formed. This cheese process produces no traditional cheese whey and maximizes the amount of UF permeate or native whey. If milk concentration is performed by microfiltration, cheese texture and flavour are not reported to be affected. With this MF-based cheese process it is possible to increase the cheese yield by 3 to 5% (Thomet and Gallmann, 2003) and at the same time to reduce processing costs.

2.6.4 Heat treatment and coagulation properties of micellar casein concentrate

Milk contains bacteria and enzymes, which both affect cheese manufacture and ripening (Pandey et al., 2003). The risk of failures during cheese manufacture and ripening can be decreased by the use of sterilized milk, using cheese milk microfiltration (1.4 μm) or high temperature heat treatment (Maubois, 2002). However, milk whey protein denaturation due to high temperature heat treatment weakens milk coagulation properties and therefore cheese milk could not be thermally sterilized. Microfiltrated casein concentrate is therefore a promising alternative for cheese manufacture. Powerful high temperature heat treatment is able to inactivate *Clostridium tyrobuturicum* spores up to 4 log units without affecting the coagulation properties of casein concentrate (Schreiber, 2001). Strongly heat treated micellar casein fraction can be used as cheese milk because strong heat treatment (100°C, 10 min) does not affect milk renneting properties or casein micelle size (Anema and Li, 2003). The reason for this is a decrease of κ-casein and β-lactoglobulin (β-LG) complexes as the concentration of β-LG is reduced, which normally weakens coagulation properties of cheese milk (Garem et al., 2000). Ultra high temperature (135°C, 1 s) (UHT) treatment has a

negative effect on the coagulation properties of milk micellar casein concentrate due to reduction of free calcium content in milk, caused by calcium phosphate precipitation.

2.7 Cheese properties

2.7.1 Effect of standardization of cheese milk protein on cheese quality

Cheese consists of a gel network and a free water fraction. Increased protein content reduces the amount of free water in cheese gel structure. The gel network contains casein and calcium salts and bound water (Kammerlehner, 1986). The free water fraction contains fat, soluble casein, byproducts from proteolysis and proteolytic enzymes, whey proteins and soluble salts with water. Free water influences proteolysis and cheese textural changes during ripening. If the rate of proteolysis is decreased, the development of cheese flavour slows down and flavour after ripening is decreased.

Microfiltrated milk causes changes in the cheese-making process if cheese quality requirements remain constant. Neocleous et al., (2002a) reported that Cheddar cheese quality was not changed if the increased milk protein content was considered in the cheese processing, shortening the cooking time and increasing the amount of rennet.

If the microfiltrated cheese milk is high temperature treated (80°C, 30 s), whey proteins are denaturated and whey protein recovery in the cheese increases. Denaturated whey proteins bind more water and cause a further increase in the cheese yield (Mead and Roupas, 2001), and also affect the cheese texture. If the milk is microfiltrated after a high temperature heat treatment (80°C, 30 s), the quality of native permeate is slightly different due to the different denaturation behaviour of individual whey proteins (Thomet et al., 2004).

The economical benefit of microfiltration in cheese manufacture is still unclear. The native whey and caseinomacropeptides (CMP) enriched cheese whey are important by-products in the cheese process including a microfiltration pretreatment. Papadatos et al. (2003) reported that microfiltration as a protein standardization method was economical for use in Cheddar and Mozzarella cheese manufacture during 10 of the 12 months of the year. Microfiltation increased processing costs but it also led to higher net revenues during the months when the prices of end products such as cream and cheese were higher. The economics of

microfiltration are dependent on individual product prices and therefore the net effect of microfiltration can vary substantially.

2.7.2 Effect of standardization of cheese milk protein on cheese ripening

Concentration of cheese milk by ultrafiltration or evaporation produces cheeses with lower moisture, slower proteolysis and decreased meltability (Acharya and Mistry, 2004). On the other hand, Mistry and Maubois (1993) and Saboya et al. (2001) reported that ultrafiltration did not have a significant negative effect on cheese ripening. It has been reported that whey proteins inhibit cheese proteolysis when ultrafiltered cheese milk is used for cheese production (Lawrence, 1989; Bech, 1993). In the case of ripened cheeses, crumby texture and bitter taste of cheese have been reported (Hinrichs, 2001). However, in the manufacture of semi-hard and hard cheeses higher salt and whey protein concentrations in the cheese are obtained by ultrafiltration. Although economically beneficial the taste, structure and functional properties of the cheese are often compromised (Mistry and Maubois, 1993). De Koning et al. (1981) reported that whey proteins slow down cheese proteolysis due to their resistance to proteolytic enzymes of rennet and starters, and thus slow down cheese ripening. The same effect was found in cheeses in which microfiltration was used as a protein standardization method and the native whey protein content of the cheese milk was increased (St-Gelais et al., 1995). Neocleous et al. (2002a) proposed three possible reasons for the decreased proteolysis. One reason could be the reduced amount of substrates for chymosin when milk non-fat dry matter is reduced. The second reason could be the larger whey proteins in the microfiltrated milk retentate. The third reason could be lower rennet content in the cheese. The amount of rennet in the cheese milk affects the residual chymosin content in the cheese and this affects the proteolysis of α_s- and β-casein. Rennet, peptidases from starters and milk plasmin together with some other enzymes such as phosphatase are responsible for cheese proteolysis (McSweeney, 2004).

When microfiltration (MF) was used for casein concentration, higher milk casein content during milk coagulation increased hardness of cheese due to the lower amount of linkages between whey proteins and caseins (Rodríguez et al., 1999). However, aroma and flavour development in MF cheeses are not affected, contrary to the case with ultrafiltration (UF) cheeses, where the content of whey proteins is higher (Bech, 1993). UF concentrates all milk proteins, which are known to act as plasmin inhibitors in cheese milk. Plasmin initiates the breakdown of β-casein into γ-casein (Ardö, 2001). Plasmin also hydrolyses α_{s1}-casein into

α_{s1}-I-casein at high pH values, as does chymosin at low pH values (Larsson et al., 2006). The concentration of plasmin inhibitors such as whey proteins and plasminogen inactivators has a negative effect on cheese flavour intensity (Benfeldt, 2006). In addition, hydrolysis of denaturated whey proteins during extensive ripening imported an atypical flavour and texture to the UF cheese (Lelievre and Lawrence, 1988). Other high molecular mass whey proteins, such as α_2-macroglobulin, have been observed to be concentrated in the MF retentate and possibly to inhibit chymosin activity during the MF cheese ripening (Neocleous et al., 2002a; Ardisson-Korat and Rizvi, 2004). Cheeses made by MF contain less residual rennet chymosin, because the dosage of chymosin can be reduced due to faster coagulation (Benfeldt, 2006). A reason for the different ratio is faster coagulation because of the higher protein content, which in turn is compensated by a reduced amount of chymosin.

2.8 Composition of native and traditional cheese whey

Separation of the whey proteins from the cheese milk into MF permeate (native whey) prior to cheese manufacture allows whey proteins to be further processed into value-added whey products such as a native whey protein concentrate as a liquid (Marcelo and Rizvi, 2008) or powder (Garem et al., 2000). MF permeate as a native whey does not contain fat, casein or casein dust nor any other by-products from the cheese manufacture (Ardisson-Korat and Rizvi, 2004). However, native whey can contain some traces of casein when membrane integrity or membrane poresize distribution may cause some loss of casein to permeate. It has been also reported that some nonmicellar individual casein monomers are dissociating from micelles and end up to native whey (Zulevska et al., 2009). Lower permeability of immunoglobulins, BSA and lactoferrin to native whey compared to permeability of α-lactablumin or β-lactoglobulin has been reported (Jost et al., 1999) but clear reason for that is still unclear (Zulevska et al., 2009). Native whey is almost free of bacterias and somatic cells when these are larger than membrane pore size (800 kDa). Cheese whey contains starter bacterias and lactic acid due to cheese starter bacteria growth during cheese cooking step. Native whey is free of caseinomacropeptides (CMP), cells, phages and thermally formed κ-casein β-lactoglobulin complexes (Maubois, 2002) which are not passed through membrane due to higher molecular size. During cheese manufacture the functionality of whey proteins decreases, reducing their biological activities (de la Fuente et al., 2002). These main differences between native and cheese whey are listed in Table 2.

Table 2. Main differences between the composition of native whey (MF permeate) and sweet cheese whey (Maubois, 2002; Ardisson-Korat and Rizvi, 2004).

Component in whey	Native whey	Cheese whey
Fat	no	yes
Cheese fines	no	yes
Casein	yes, traces	yes
Caseinomacropeptides (CMP)	no	yes
Bacteria	no	yes
Somatic cells	no	yes
Lactic acid	no	yes
κ-casein and β-lactoglobulin complexes	no	yes
Cheese chymosin	no	yes
Immunoglobulins	yes, minor amounts	yes

Transfer of whey total solids into the microfiltration (MF) permeate changes the content of whey protein in the cheese milk, since the permeability of the main whey protein components into the MF permeate depends on the concentration factor (CF) (Outinen et al., 2008) and also on the retention of large proteins. Retentions of lactoferrin (LF), bovine serum albumin (BSA) immunoglobulins (Ig) and proteose-peptone components in the MF retentate were reported by Jost et al. (1999). In their study, skimmed milk was microfiltered with a ceramic 0.14 μm membrane to CF 3 and diafiltered to CF 6, after which the MF retentate still contained 5% of non-casein protein. Nelson and Barbano (2005) reported 5% residual whey proteins in MF retentate at CF 27, whereas Samuelsson et al. (1997b) measured 10% retention of non-casein nitrogen at CF 3.

The amount of whey components in the traditional sweet whey does not depend on the extent to which the components are recovered from the cheese milk in the whey. Ardisson-Korat and Rizvi (2004) observed that the recovery of whey proteins in whey decreased 14.8-15.8% in whey with increasing CF from CF 6 to CF 9 with 0.1 μm membranes. Recovery of whey proteins to press whey increased proportionally and at the same time there were no significant differences in the recovery of casein to whey (0.9-1.13%). Brandsma and Rizvi (2001) reported 11.5% recovery of skimmed milk whey proteins into chymosin whey using CF 8 retentate. Increase of whey protein and fat of total solids (TS) in whey has been reported by St-Gelais and Haché (1995) and St-Gelais et al. (1995).

2.8.1 Effect of microfiltration on whey processing

Native whey production reduces the amount of cheese whey and changes its composition (Maubois, 2002). The influence of microfiltration (MF) on cheese whey has not been thoroughly studied and the influence of cheese milk microfiltration on native and cheese whey mass balances is still unclear. Govindasamy-Lucey et al. (2007) studied the effect of microfiltration (MF) on the structure, quality and yield of pizza cheese, and on the quality of whey but in this study the cheese whey usability compared to the reference whey was not analyzed. Thomä and Kulozik (2005) reported that MF cheese whey contains more caseinomacropeptide (CMP) than traditional cheese whey and MF whey can be further processed to retain pure CMP fractions. Higher protein level in cheese milk reduces cheese whey, depending on the concentration factor (CF) during microfiltration. Microfiltration permeate contains whey proteins which are normally transferred to cheese whey. The CMP content of cheese whey is much higher if whey proteins are partially removed to the MF permeate.

2.8.2 Biological and functional properties of proteins from native whey

The biological and functional properties of whey proteins are important due to their use in the food and pharmaceutical industries. In the food industry, whey proteins are used to create food matrix, bind water or to create protein gels. Therefore solubility, water binding, gelling, foaming and emulsifying properties of individual whey proteins, modified and hydrolyzed whey proteins are attracting more attention (Foegeding et al., 2002). More attention has also been paid to the biological properties of food and therefore the influence of whey proteins on human nutrition has become more critical (Luhovyy et al., 2007). The main whey proteins and biological properties as well as the contents in milk are presented in Table 3. The functional properties of the individual whey proteins are summarized in Table 4.

Table 3. The main whey proteins of milk, their contents and biological functions.

Main whey proteins	Content in milk	Biological function	Reference
β-lactoglobulin (β-LG)	3.2 g/L	Stimulate glutathione synthesis	(McIntosh et al., 1995)
α-lactalbumin (α-LA)	1.2 g/L	Support biosynthesis of lactose	(De Wit, 1998c)
Bovine serum albumin (BSA)	0.4 g/L	Binds insoluble free fatty acids	(Harzer and Haschke, 1989)
Immunoglobulin G (IgG)	0.8 g/L	Passive immunity	(Larson, 1989)
Bioactive whey proteins			
Lactoferrin (LF)	0.2 g/L	Antimicrobiological activity, bind iron	(Nichols and Kee, 1990; Jenssen and Hancock, 2009)
Growth factors	<1.2 mg/L	Impact on immune system and growth	(Purup et al., 2007)
Lactoperoxidase	0.03 g/L	Part of the bactericidal system	(Nichols and Kee, 1990)
Proteose-peptones	>1.0 g/L	Gastrointestinal adsorption of calcium	(Kitts and Yuan, 1992)
Caseinomacropeptides (CMP)	0.18 g/L	Contains no aromatic amino acids, mineral adsorption enhancing, prebiotic	(Brück et al., 2003a; Brück et al., 2003b)
Non-glycosylated caseinomacropeptides (ngCMP)	0.09 g/L	Anticariogenic, immunomodulating activities	(Thomä-Worringer et al., 2006; Kreuß and Kulozik, 2009)
Glycosylated caseinomacropeptides (GMP)	0.08 g/L	Bioactivity: function of cell membrane and membrane receptors in brain development, interacts with toxins, viruses and bacteria, contains five different mucin-type carbohydrate chains and sialic acids	(Saito and Itoh, 1992; Kreuß and Kulozik, 2009)

Table 4. The functional properties of the main whey proteins and their other features.

Whey protein	Functional properties	Other features	Reference
β-lactoglobulin (β-LG)	Foaming, gelation, lipid binding	Most allergenic bovine milk protein	(Tolkach and Kulozik, 2004)
α-lactalbumin (α-LA)	Emulsifying, viscosity	High amount of essential amino acids	(Tolkach and Kulozik, 2004; El-Shibiny et al., 2007)
Bovine serum albumin (BSA)	Flavour and compound binding	-	(Tan and Siebert, 2008)
Caseinomacropeptides (CMP)	Emulsifying, foaming properties, mouthfeel	Hypoallergenic, lacks aromatic amino acids	(Abd El-Salam et al., 1996; Kulozik and Guilmineau, 2003)

Whey proteins are a special group of proteins due to their different functional, physiological and biological properties (Fox, 2001). The functional properties of whey proteins are affected by the proteins themselves, but also by the process conditions and by environmental factors such as pH, temperature and ionic strength (De Wit, 1988).

The main whey protein is β-lactoglobulin (β-LG), representing 80% of total milk whey proteins. β-Lactoglobulin has excellent foaming and gelation properties and may be capable of binding fatty acids and lipids and some other small hydrophobic molecules such as retinol. However, β-LG is one of the most allergenic bovine milk proteins for humans (El-Agamy, 2007). The second main whey protein is α-lactalbumin (α-LA), which has significantly

higher thermal stability against unfolding and lower gel formation properties compared to β-LG (Tolkach and Kulozik, 2004) when it is present in mixed matrices such as milk. α-Lactalbumin is a metallo-protein and it has high affinity for calcium (Ca^{2+}) ions, which means that both calcium-free (apo α-lactalbumin) and calcium-bound (holo α-lactalbumin) forms of α-LA exist (Thompson and Brower, 1989). These apo- and holo α-lactalbumins have different denaturation temperatures and numbers of denaturation step. Apo denatures at 41-43°C with a 2-step denaturation reaction and holo at 66-67°C with a 3-step reaction route (Apenten, 1995), which means that the denaturation temperature of β-lactoglobulin (78-84°C) may be higher in pure solutions (Kessler, 2002b). However, denaturation behaviour of whey proteins is dependent also of many other factors like pH value, lactose and protein concentration in solution (Kessler, 2002b). α-LA contains a high amount of essential amino acids, which are needed in muscle protein synthesis, and it also stimulates human brain serotonin activity due to its high tryptophan (Trp) content (Markus et al., 2000).

The caseinomacropeptide (CMP) is considered to be a sweet cheese whey constituent, but it is actually a hydrophilic C-terminal part of κ-casein, which is cleaved by chymosin during milk coagulation. CMP is water soluble and it ends up in cheese whey. CMP has a unique amino acid composition, combining a high threonine (Thr) content with the total absence of all aromatic amino acids, and therefore pure CMP can be used as an ingredient in diets for phenylketonuria (PKU) patients (Abd El-Salam et al., 1996). CMP has few functional properties, but it has a positive influence on the mouthfeel and flavour of food because it is heat stable and does not release off-flavours during heat treatment. CMP is hypoallergenic, it is easily absorbed and digested, and it has a strong anti-infective effect as well as high nutritive value (Takahashi et al., 1992). It can be used as a food structuring agent because of its emulsifying and foaming properties (Kulozik and Guilmineau, 2003).

Bovine serum albumin (BSA), immunoglobulins (Ig), growth factors and other minor whey proteins have an important biological significance for the human immune system, but their functional properties in food applications are far less well known. Immunoglobulins such as the other large whey proteins are relatively thermolabile, and even moderate heat treatments in dairy processes denaturate these proteins (Cao et al., 2007), reducing their technological potential.

2.9 Aims of this study

The main objective in this thesis was to compare different cheese milk pretreatment methods from the point of view of cheese and whey. Microfiltration (MF) of milk with a membrane having a pore size of 0.05-0.2 μm has an influence on cheese milk coagulation, cheese yield and cheese ripening as well as on whey quality and on the functional properties of the whey product. These influences were studied and evaluated for their significance for cheese, whey and milk component usability. Effects of reduced lactose, whey protein and salt content in cheese milk coagulation together with cheese milk protein standardization have not been studied previously. In his study it was assumed that microfiltration in cheese process increases cheese yield and improves cheese whey functionality.

Microfiltration technology has been developed further by membrane manufacturers and one object of this study was to measure and compare the energetic performance of different microfiltration membranes in skimmed milk microfiltration. It was also hypothesized that polymeric microfiltration membranes have poorer performance in whey protein separation in skimmed milk filtration compared to ceramic membranes. Does microfiltration affect milk coagulation or cheese quality and ripening when the cheese milk ash, whey protein and lactose concentrations are decreased? It was suspected that α-lactalbumin and β-lactoglobulin were transferred better from milk to cheese when the amount of these components in cheese milk were decreased and at the same time cheese whey contained higher amounts of CMP of total protein. It was also believed that microfiltration as a protein standardization method improved cheese yield as much as ultrafiltration and high temperature heat treatment. One hypothesis was that whey protein concentrate made from native whey had better functional properties than cheese whey protein concentrate powders. To resolve the main questions, seven studies were performed, with the specific aims described below:

1. Measurements and calculations of filtration performance with ceramic, polymeric spiral wound and hollow fiber membranes were made to estimate the aspects of microfiltration (MF) technology affecting milk microfiltration. Trials with hollow fiber membranes were made to detect the influence of filtration parameters on permeate flux, β-lactoglobulin mass flux and whey protein retention values.

2. The effects of decreased milk lactose and ash content on cheese ripening and sensory quality were evaluated. Ripening of cheese produced with or without microfiltration (MF) was studied (**I**).

3. The influence of MF cheese milk on the recovery yield of milk components from milk to whey with different CF values (MF) was studied. Retention coefficients for the main whey proteins α-LA and β-LG with different concentration factors (CF) were analyzed (**II**),

4. The effect of milk modification on milk coagulation properties was studied. The influence of reduced milk lactose, whey protein and salt content on cheese milk coagulation properties was also examined (**III)**,

5. Recovery yields of milk components to cheese were determined from micro- (MF) and ultrafiltered (UF) and high temperature heat treated (HH) modified milks. The influence of cheese milk protein standardisation using polymeric MF and UF on Edam cheese manufacture, ripening and functional characteristics was studied (**IV**),

6. The effects of three cheese milk pretreatment methods, microfiltration (MF), ultrafiltration (UF) and high temperature heat treatment (HH), on cheese whey amount and quality were determined. Amino acid compositions of native and cheese whey protein concentrate powders made with MF, UF and HH whey were evaluated (**V**).

7. The effects of drying methods on whey protein concentrate (WPC35) functionality (solubility, viscosity, gelation, foaming properties, emulsification and water-holding capacity) of native whey (microfiltration permeate) were compared to those on traditional cheese whey (**VI**).

3 MATERIALS AND METHODS

This study consists of the publications I-VI. Detailed materials and methods are described in each publication. Here only a brief summary of the materials and methods used is given.

3.1 Raw materials

3.1.1 Milk and whey

In studies I, II and III the whole milk was obtained from a local dairy (Valio Ltd., Helsinki, Finland) and in studies IV-V skimmed and pasteurized milk (72±2°C for 15 s) was received from a cheese factory (Valio Ltd., Lapinlahti, Finland). In study VI raw milk for the MF filtrations was obtained from a local farm and whey from the cheese factory (Valio Ltd., Lapinlahti, Finland). Before microfiltration (0.1 μm) milk was skimmed by microfiltration (1.4 μm) in study VI or with a separator in studies I-V. In study VI the milk was not pasteurized. In hollow fiber filtration trials pasteurized (71°C, 20 s) and skimmed milk was obtained from a local dairy (Staatliche Molkerei Weihenstephan GmbH, Freising, Germany). UF permeate for skimmed milk MF diafiltration was obtained by using UF to deproteinize MF permeate in studies I and III.

3.1.2 Filtration equipment, filtration parameters and heat treatments

All filtrations were carried out in batch mode using different concentration factors (CF) depending on the aim of the filtration. The first filtrations were performed with old (uniform transmembrane pressure, UTP) and new type (gradient permeability, GP) of ceramic membranes and the last filtrations were done with new type of polymeric membranes after performance tests. Used polymeric spiral wound and hollow fiber microfiltration membranes were selected by testing different membranes on laboratory scale and the selected membranes had best retentions for casein and highest permeate flux values. Total concentration factor of filtration was the CF value of microfiltration multiplied by the diafiltration CF value in cases where diafiltration was used. Concentration factor was calculated as described in equation 6.

$$\mathrm{CF}\,(-) = \left(\frac{\text{feed}\quad(\mathrm{L})}{\text{retentate}\quad(\mathrm{L})}\right) \quad \mathrm{x} \quad \left(\frac{\text{diafiltration feed}\quad(\mathrm{L})}{\text{diafiltration retentate}\quad(\mathrm{L})}\right) \tag{6}$$

Permeability of β-lactoglobulin (β-LG) and permeate fluxes values through 500 kDa hollow fiber (HF) membranes (RomiPro™, PM500, 1"x18", 1.1, Wilmington, Koch Ltd, USA) was measured at a concentration factor (CF) value of 1 with a laboratory filtration unit (Technical University of Munich, Freising, Germany), using a total filtration area of 0.092m^2. Transmembrane pressure (TMP) varied from 0.36 to 0.83 bar and tangential flow velocities from 2.0 to 3.5 m/s at 55±2ºC. The hollow fiber membrane (HF) showed retentate inlet pressures of 0.65 to 1.24 bar and retentate outlet pressures 0.0 to 0.68 bar depending on the tangential flow rates and TMP values.

Transmembrane pressure (TMP) during filtration has a major impact on membrane performance. Mean TMPs for polymeric MF (FR-2B-3838), UF (HFK-131, type 2540-30D; 5838-HFK-131-NYT) and hollow fibre (RomiPro™, PM500) membranes were calculated using equation 7 and for ceramic (Membralox P19-40) membranes using equation 8. However, ceramic GP membrane (Membralox P37-30) TMP values were calculated from retentate outlet and permeate outlet pressures because of the membrane gradient structure.

$$\text{TMP (bar)} = \left(\frac{\text{retentate in (bar)} + \text{retentate out (bar)}}{2} \right) \quad (7)$$

$$\text{TMP (bar)} = \left(\frac{(\text{retentate in (bar)} - \text{permeate in (bar)}) + (\text{retentate out (bar)} - \text{permeate out (bar)})}{2} \right) \quad (8)$$

Microfiltration of skimmed milk was carried out in the studies I and III in a Tetra Alcross MFS-1 filtration unit (Tetra Pak, Højbjerg, Denmark). Ceramic uniform transmembrane pressure (UTP) membrane (0.1 μm, 0.24 m^2, Membralox P19-40, Pall Co., Bazet, France) was used in the filtration unit at a transmembrane pressure (TMP) of 0.3 bar at 55±1˚C. In study VI the ceramic gradient permeability (GP) membrane 0.1 μm 0.36 m^2 (Membralox P37-30, Pall Co., Bazet, France) was used instead of the UTP membrane due to of larger filtration area. In study VI milk skimming was performed using the Tetra Alcross MFS-1 plant with a 1.4 μm, 0.24 m^2 membrane (Membralox P19-40, Pall Co., Bazet, France) and a TMP of 0.3 bar at 50±1ºC. In study V the Tetra Alcross MFS-1 plant with 0.8 μm, 0.24 m^2 membrane (Membralox P19-40, Pall Co., Bazet, France) was used for whey skimming with 0.3 bar at 50±1ºC. In study IV skimmed milk microfiltration was carried out at 50±5ºC using four spiral wound (SW) polymeric polyvinylidene fluoride (PVDF) 800 kDa membranes (FR-2B-3838,

Synder Filtration, Vacaville, USA) in the Niro Combi Plant filtration unit (Niro A/S, Soeborg, Denmark) with total a filtration area of 28.2 m^2 and TMP of 0.7 bar.

Ultrafiltration of sweet whey (study VI) was performed with a DDS Labstak® M37/38 unit (DDS AS, Silkeborg, Denmark) using six 10 kDa membranes (M37, GR81PP, total membrane area 0.66 m^2). Ultrafiltration of native whey (studies I, II and III) was performed with the ProScale™ unit (Millipore S.A., Molsheim, France) using a 10 kDa membrane (HFK-131, type 2540-30D, 2.4 m^2, Wilmington, Koch Ltd, USA) and 2.5 bar TMP at 20±1ºC. Permeate from native whey ultrafiltration was used as a diawater for microfiltration in studies I and III. Ultrafiltration of skimmed milk in studies IV and V was carried out at 50±5ºC in the Niro Combi Plant filtration unit with 2.5 bar TMP using 10 kDa spiral wound (SW) polyethersulfone (PES) membranes (5838-HFK-131-NYT, Koch Membranes, Wilmington, USA) with a total filtration area of 100.2 m^2. Whey ultrafiltration in study VI was performed using the ProScale™ unit using the same 10 kDa membrane and operation parameters as with native whey and whey described above.

For spiral wound (SW) microfiltration the retentate inlet pressure was 1.1 bar, retentate outlet 0.3 bar, meaning 0.8 bar pressure drop over the membrane. This 0.8 bar pressure drop created ca. 0.5 m/s tangential flow rate near the membrane surface. Ultrafiltration retentate inlet pressure was 3.0 bar and outlet pressure 2.0 bar in studies I to III. Ultrafiltration membranes in studies IV and V had retentate inlet pressure 4.0 bar and retentate outlet pressure 1.0 bar. In studies I to V all UF trials were performed with 2.5 bar TMP. In study VI flat sheet UF (DDS Labstak® M37/38 unit) retentate inlet pressure varied from 5.5 to 3.8 bar and retentate outlet pressure from 5.2 to 3.5 bar, resulting in TMPs between 4.1 and 5.2 bar. Ceramic uniform transmembrane pressure (UTP) membranes (0.1 and 1.4 μm) had retentate inlet pressure 3.5 bar, outlet pressure 2.0 bar and permeate inlet pressure 3.1 bar and outlet pressure 1.7 bar. Pressure drop over the UTP membranes was 1.5 bar, which resulted in a tangential flow rate of 6.1 m/s. For GP membrane it was assumed that TMP levels in the retentate inlet and outlet were identical when the pressure drop over the membrane was 1.95 bar. This pressure drop caused a tangential flow rate of 6.1 m/s in the GP membrane channel. GP membrane had retentate inlet pressure 4.0 bar, outlet pressure 2.1 bar and permeate inlet pressure 1.8 bar and outlet pressure 1.8 bar.

High temperature heat treatment (HH) of milk at 93°C for 15 s in study IV was performed using a plate heat exchanger (Alfa Laval, C8-SR, Lund, Sweden). Traditional pasteurization

of milk at 72°C for 15 s was performed in study I with a EuroCal 5FGH (Fischer Mascinen- und Apparatebau AG, Austria) and in study IV with an APV Pasilac H07 equipment (APV Pasilac AS, Kolding, Denmark).

3.1.3 Equipment cleaning, water flux measurement and cleaning of membranes

Cheese vats, all pipes and tanks were cleaned after 10 min tap water flushing in a two step cleaning-in-place (CIP) procedure with 1.0% (v/v) NaOH at 75°C for 30 min and 1.0% (v/v) HNO_3 for 20 min at 70°C. All membranes and filtration plants were cleaned according to membrane and equipment manufacturer instructions. Membrane filtration plants and all equipment were cleaned if over 24 h had elapsed since the previous cleaning procedure. After filtration and after the cleaning procedure, filtration plants were flushed with tap water at 10°C for 20 min.

Hollow fiber (HF) membranes were cleaned in a three step procedure with 1% (v/v) Ultrasil 14 caustic (Ecolab, Düsseldorf, Germany) at 50°C for 40 min, with 0.5% (v/v) HNO_3 acid (Staub & Co., Nürnberg, Germany) at 50°C for 30 min and with 1.0% (v/v) Ultrasil 14 (Ecolab, Düsseldorf, Germany) at 50°C for 30 min.

After flushing, ceramic membranes (0.1 μm, 0.8 μm and 1.4 μm) were cleaned in a three step procedure with 1.0% (v/v) cleaning Divos 124 caustic (JohnsonDiversey Ltd., Turku, Finland) at 80°C for 40 min, with 0.5% (v/v) nitric (HNO_3) acid (Nitric acid 65% GR, Merck KgaA, Darmstadt, Germany) at 50°C for 25 min and with 1.0% (v/v) Divos 124 at 80°C for 30 min.

Polymeric spiral wound membranes in the Niro Combi Pilot Plant were cleaned in a three step procedure with 0.8% (v/v) F80 FILTER HE and 0.4% (v/v) F93 FILTER EN enzymatic caustic (Farmos Ltd., Turku, Finland) at 45°C for 40 min, with 0.4% (v/v) F91 FILTER VH acid (Farmos Ltd., Turku, Finland) at 40°C for 20 min and 0.8% (v/v) F80 FILTER HE caustic (Farmos Ltd., Turku, Finland) at 45°C for 30 min. Polymeric plate and frame (M37, GR81PP) and polymeric spiral wound (HFK-131, type 2540-30D) filters were cleaned in a three step procedure with 0.8% (v/v) Divos 2 acid (JohnsonDiversey Ltd., Turku, Finland) at 50°C for 30 min, with 0.8% (v/v) Divos 108 and 0.6% (v/v) Divos 80-6 enzymatic caustic (JohnsonDiversey Ltd., Turku, Finland) at 45°C for 40 min and with 0.6% (v/v) Divos 2 acid

at 45°C for 30 min. After the cleaning steps, intermediate flushing with tap water was performed for 15 min.

The cleaning effect was controlled by measuring the membrane water flux under standard conditions. When the water flux was >10% below the previous membrane water flux record, the cleaning procedure was repeated.

3.2 Coagulation tests

In study III, different amounts of $CaCl_2$ 34% (w/w) solution (Tetra Chemicals AB, Sweden) together with the 1:100 diluted chymosin (MIC Coagulant 600, Chr. Hansen, Denmark) were added to milk samples before the coagulation tests. Coagulation tests were performed with a Formagraph 20 equipment (Foss Electric, DK-3400, Hillerød, Denmark). Milk samples (10 mL) were renneted at 32°C. Rennet clotting time (RCT), curd firmness 40 min after rennet addition (A40) and the time required to achieve a curd firmness of 20 mm (K20) were determined. The parameter (K20-RCT) was calculated in order to evaluate the development of curd firmness rate.

3.3 Cheese milk pretreatment and cheese manufacture

In studies I and III cheese trials were performed as presented in Figure 7. The cheese whey (see Figure 7) was further processed and used as raw material for study II. Milk pretreatments in the trials 2-5 aimed to increase milk casein concentration by a factor of 1.4 (except in study IV where the final CF was 1.0 in trial 3) and to reduce the proportion of whey proteins. Total CF for trials 1 to 5 was 1.0; 1.4; 4.0; 10.8 and 10.8, respectively. In trials 4 and 5 the filtration procedure was similar, except that in trial 5 recombination of milk was performed with water and in trial 4 with UF permeate.

In studies I and III a lower amount of chymosin was used for concentrated milks except in trial 3 of study III (Figure 7), which had the same protein content as the reference milk, but less chymosin was added. Starter, $CaCl_2$ and $CuSO_4$ additions were carried out in relation to the calculated casein content. In study V rennet, starter and $CaCl_2$ additions were similar in each trial.

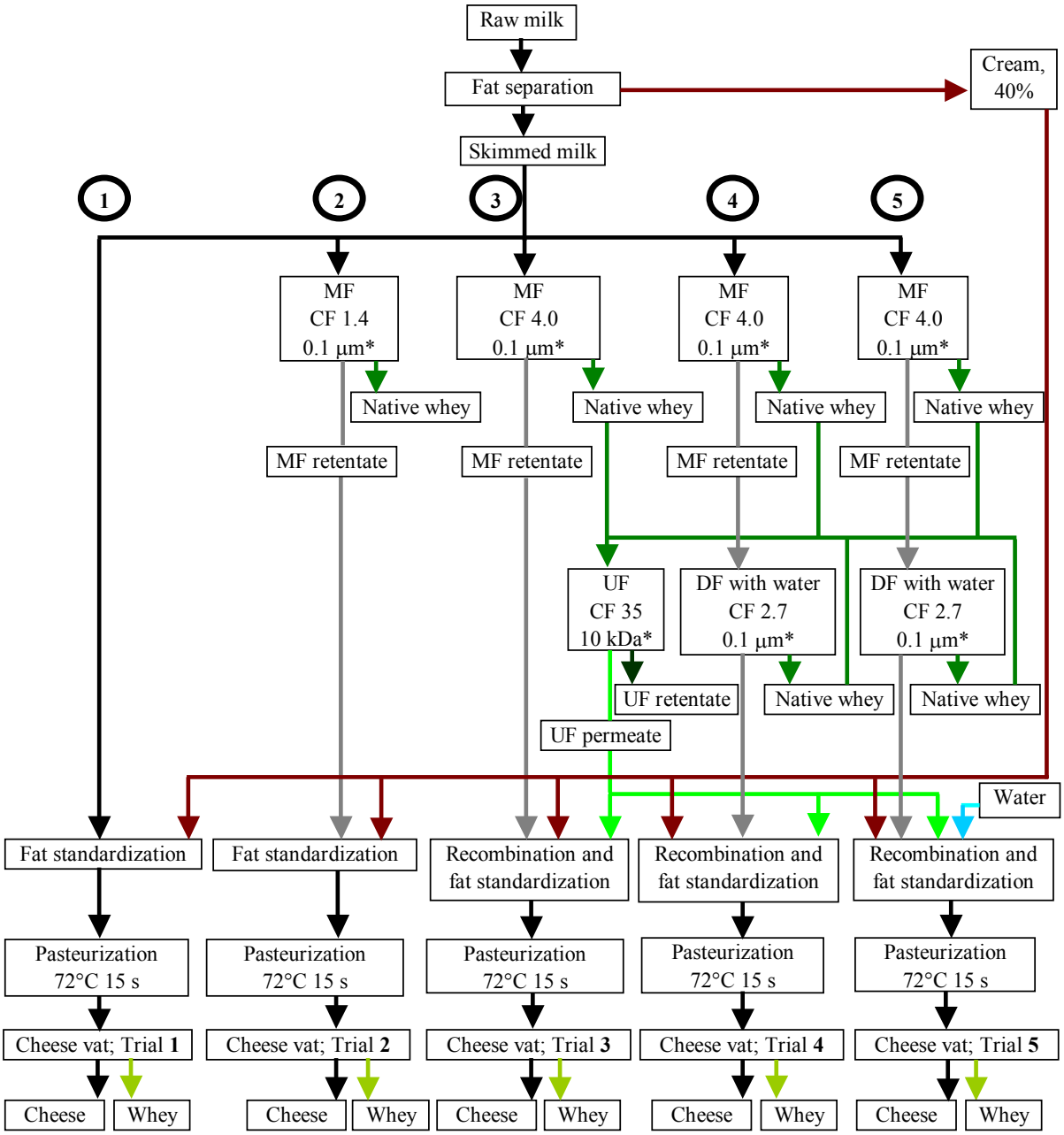

Figure 7. Process flow chart of the trials 1, 2, 3, 4 and 5 in studies I and III. The target for fat/protein ratio in standardization was 0.9 and for vat milk recombination the protein target was 4.2% in Trials 3 to 5. MF = microfiltration, UF = ultrafiltration, DF = diafiltration in microfiltration, CF = concentration factor, * = membrane pore size or cut-off value.

A cheese and whey process flow chart of studies IV and V is presented in Figure 8. Cheese whey (see Figure 8) was further processed to use as raw material for study V. Protein contents of microfiltrated and ultrafiltrated milks were increased from 3.5% to 4.2%. No filtration as a pretreatment was made for reference milk and for high temperature treated (HH) milk.

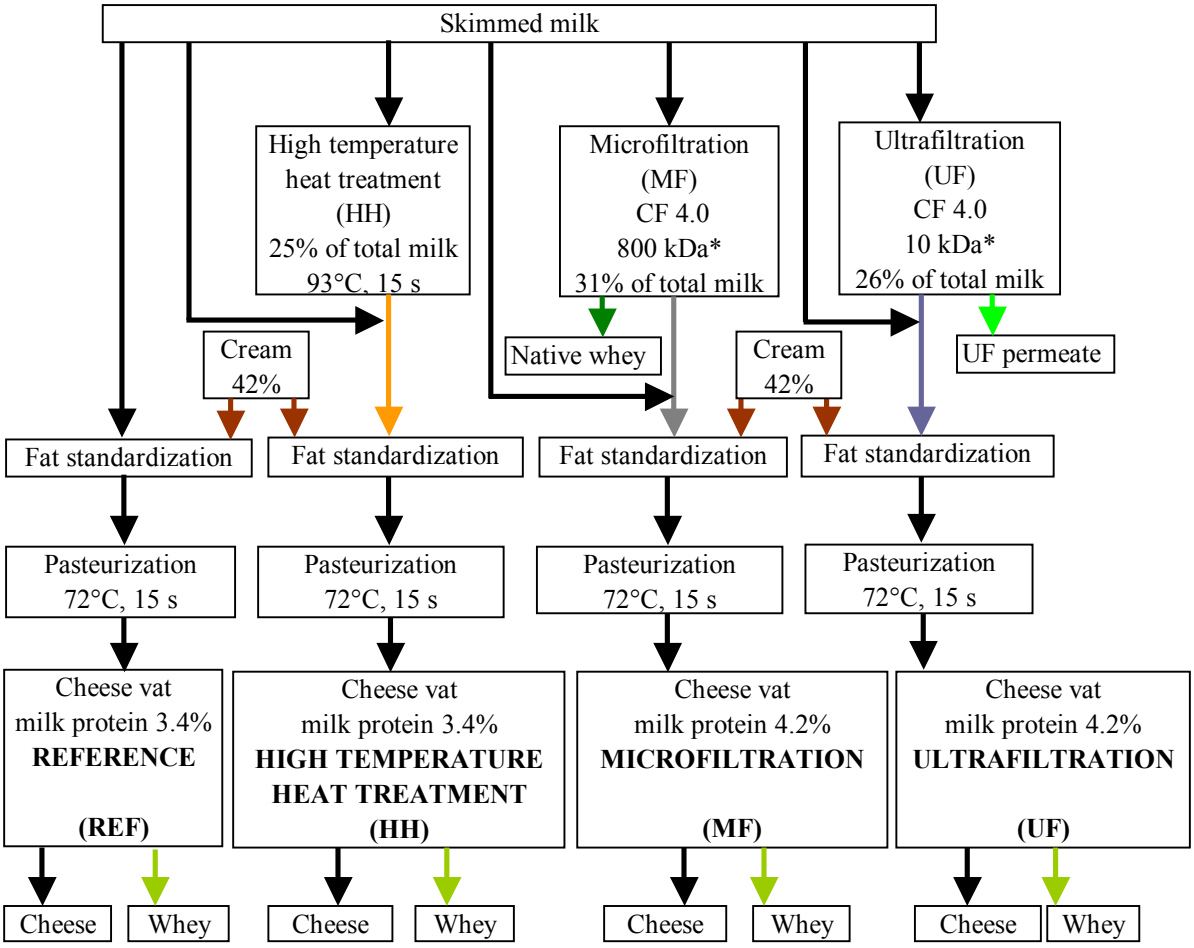

Figure 8. Process flow chart of the trials REF, HH, MF and UF in study IV. The target for fat/protein ratio in standardization was 0.8 and for MF and UF vat milk protein target was 4.2%. REF = reference, HH = high temperature heat treatment, MF = microfiltration, UF = ultrafiltration, CF = concentration factor, * = membrane pore size or cut-off value.

3.4 Whey process

In study I the cheese whey was obtained from the cheese process and whey was used without any further processing for study II. In study V the cheese whey from the cheese process and the native whey from microfiltration of milk were further processed to obtain powders of whey protein concentrates (WPC) using the process flow chart presented in Figure 9. WPCs were dried as powders from the UF retentates with a freeze dryer (GWB Edwards, Crawley, Great Britain) in studies V and VI and with a spray dryer (Niro Atomizer P 6.3, Niro A/S, Denmark) in study I.

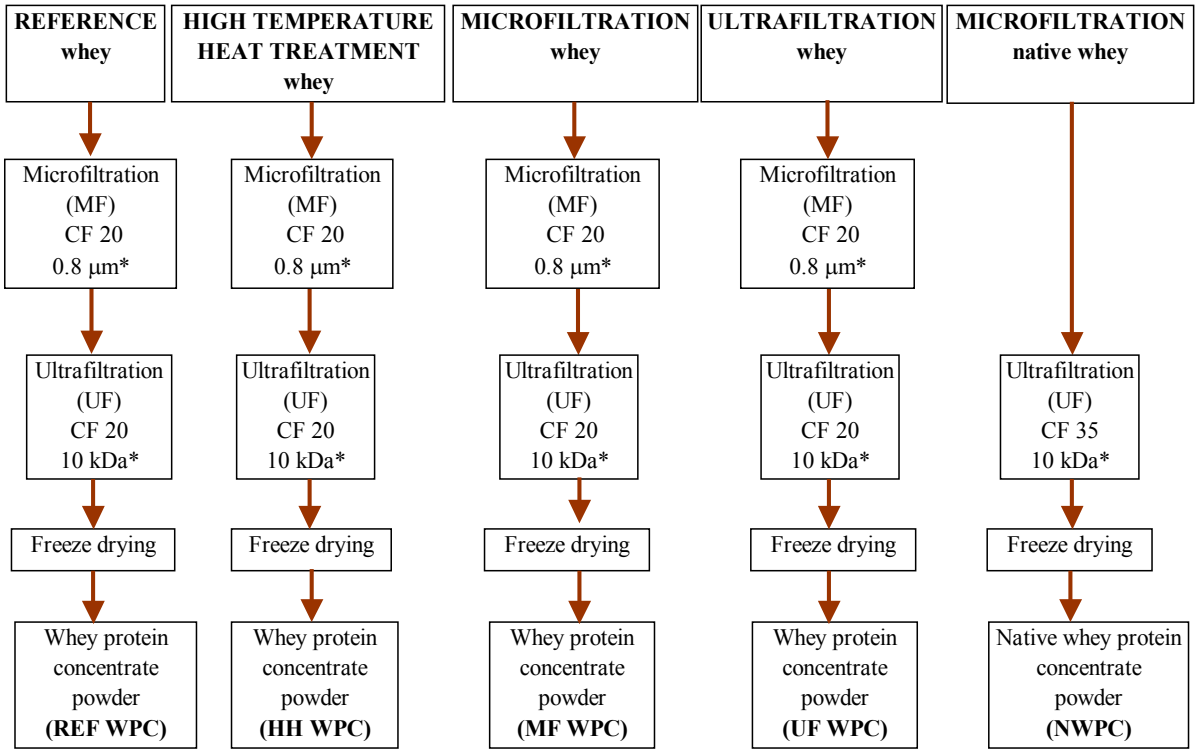

Figure 9. Process flow chart for reference (REF WPC), high temperature heat treatment (HH WPC), microfiltration (MF WPC), ultrafiltration (UF WPC) and native whey (NWPC) types of whey protein concentrate powder produced in study V. CF = concentration factor, * = membrane pore size or cut-off value.

Functionality tests of the whey protein were performed with whey protein concentrate (35% protein of total solids, w/w) (WPC 35) powders in study VI. The flow charts of these processes are presented in Figure 10. The powder (Proval 35, Valio Ltd, Lapinlahti, Finland) of industrial whey protein concentrate WPC-SD was obtained from an industrial process.

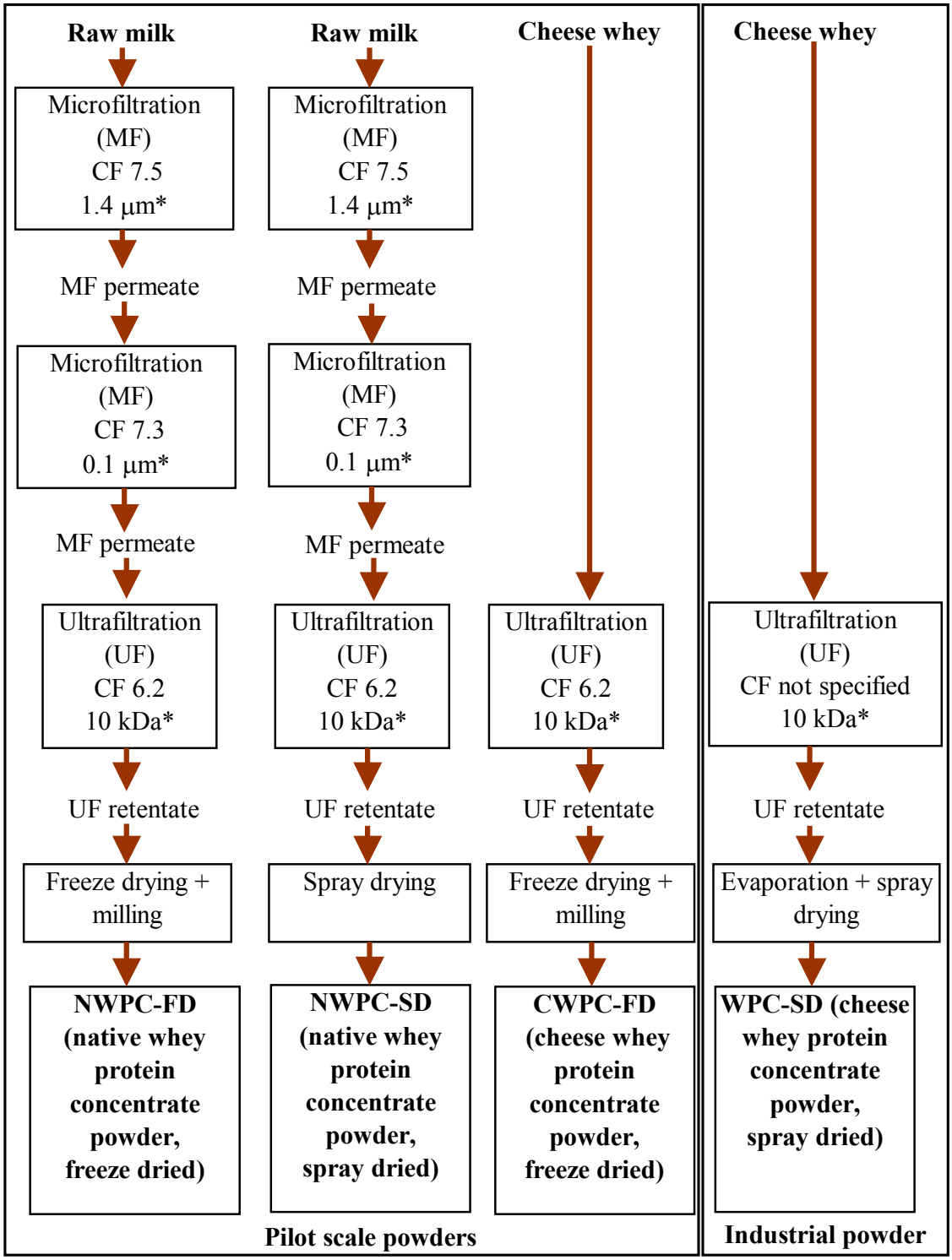

Figure 10. Process flow chart for native whey protein concentrate powder – freeze dried (NWPC-FD), native whey protein concentrate powder – spray dried (NWPC-SD), cheese whey protein concentrate powder – freeze dried (CWPC-FD) and cheese whey protein concentrate powder – spray dried (WPC-SD) types of whey protein concentrate powders (35% total protein of total solids) produced in study VI. MF = microfiltration, UF = ultrafiltration, CF = concentration factor, * = membrane pore size or cut-off value.

3.5 Analytical methods

3.5.1 Analyses of milk, whey, WPC powder and cheese samples

Samples were taken from cheese milk, whey, WPC powder, fresh cheese after pressing and from cheese after ripening. Total solids (TS) was analysed from all samples with IDF 21B:1987. Water content was calculated after total solids measurement (IDF 21B:1987) as 100 – total solids content (%). Casein nitrogen was analysed with IDF 29:1964/ISO 8968-1 and IDF 20-1:2001. Non-protein-nitrogen (NPN) was determined with IDF 20-4:2001. NPN was converted to protein equivalent (NPN-P) by multiplying by 6.38. Total nitrogen (TN) content of milk and whey was analysed using ISO 8968-1 and IDF 20-1:2002; for cheese ISO 8968-2 and IDF 20-2:2002; for WPC powder IDF 20-4:2002. Total protein content was obtained by multiplying TN by a factor of 6.38. Whey protein (WP) was calculated as follows: WP = [TN – (CN – NPN)] x 6.38. The fat contents of milk was obtained using IDF 1C and 16C:1987 and of cheese using ISO 1735 and IDF 5:2004. Fat content of whey and WPC powders were analyzed with IDF-1D:1996 and IDF-5B:1986, respectively. The lactose contents of milk, whey and cheese were analysed with IDF 79-2:2002. Whey proteins α-LA, β-LG, ngCMP and GMP were determined with reverse phase high pressure liquid chromatography (RP-HPLC) (Thomä et al., 2006). The proteins of the samples for CMP were precipitated in 6% (study VI) and 8% (study II and V) trichloroacetic acid, centrifuged and analyzed. Calibrations of the chromatographic system for the quantitative analysis were carried out by means of external standards and measured by with a UV detector at 280 nm. The purified preparations for the calibrations were α-LA L-0610 (Sigma) and β-LG (L-0130, Sigma). Whey protein analysis was performed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (1970), using ready-made 18% Tris-HCl polyacrylamide gels (Bio-Rad, Hercules, USA). Protein bands (10 μg load) were stained with Coomassie G-250 (GelCode Blue Stain Reagent, Pierce, USA) and compared with IgG (Perstorp Biolytica, Lund, Sweden), BSA and LF standards (Sigma, St. Louis, Missouri, USA). The pH of the milk and cheese was measured with Knick SE 104 (study I) or WTW 330 (study IV) combination puncture electrodes. Salt contents of cheese were measured by potentiometric chloride titration (IDF 88/ISO 5943:2006). Ash contents of whey and WPC powders were analyzed with IDF 154:1992. Calcium contents of milk and WPC powders (study II) were measured with ICP-MS -equipment (Inductively Coupled Plasma Mass Spectrometry, Elan 6100 Perkin Elmer, Waltham, USA). Titratable fatty acids (TFA) and lactic acids in cheese were measured using a titration method (Moisio and Heikonen, 1996)

and with IDF 69B:1987, respectively. Cheese carboxylic acids were measured with a gas chromatography -method (De Jong and Badings, 1990) and β-casein with fast protein liquid chromatography equipment (Syväoja, 1992) in study I. Amino acids and tryptophan (Trp) contents of cheese milk (study IV) and WPC powders (study V) were determined with AnalyCen AB (Lidköping, Sweden) according to SFS standards (SFS, 2005a; SFS, 2005b). Cheese moisture in the non fat substance (MNFS) was calculated with the formula MNFS (g/kg) = (100-TS) / (100-Fat) x 1000. Fat on a dry basis (FDB) was calculated with the formula FDB (g/kg) = (Fat/TS) x 1000.

3.5.2 Sensory analyses of cheese

In study IV cheeses were analysed in the Sensory Analyses group (Valio Ltd., R&D, Helsinki, Finland) at 14±1°C under normal light using triangle and consumer tests. Each cheese was evaluated by 15 trained panelists in order to detect differences between the cheeses. Each panelist was asked to choose the different cheese among three cheeses. The consumer test with 14 untrained panelists was used to determine the pleasantness of different sensory properties (overall pleasantness, structure, flavour, appearance) using a rating from 4 to 10 (4=not good, 10=excellent).

3.5.3 Textural analyses of cheese

Textural properties were analysed with a TA.XTplus texture analyser (Stable Micro Systems Ltd, Surrey, UK) in the IV. Hardness and cohesiveness were tested by compressing a cheese sample twice, with an interval of five seconds, between two metal plates into 25% of its original height. Springiness and resilience were analysed by compressing the cheese sample to 70% of its original height. Cheese samples were cylindrical (23 mm in diameter and 20 mm in height). Six repeats were performed for each cheese sample. Samples were temperated at 14°C for at least 16 hours before the analyses.

3.5.4 Calculations for cheese yield and recovery of milk components

The most important factors in cheese manufacture are cheese properties and recovery yield of milk components in the cheese. In study IV, cheese yield (CY_v and CY_r) and adjusted cheese yield (ACY_v and ACY_r) from vat and raw milk were calculated using equations (9), (10), (11) and (12) respectively. In study I only CY_v and ACY_v were calculated.

$$CY_v\ (\%) = \frac{\text{mass of cheese (kg)} \times 100}{\text{mass of vat milk (kg)}} \quad (9)$$

$$CY_r\ (\%) = \frac{\text{mass of cheese (kg)} \times 100}{\text{mass of raw milk (kg)}} \quad (10)$$

$$ACY_v\ (\%) = \frac{\text{mass of cheese (kg)} \times 100}{\text{mass of vat milk (kg)}} \times \frac{46}{\text{cheese moisture (g/100g)}} \quad (11)$$

$$ACY_r\ (\%) = \frac{\text{mass of cheese (kg)} \times 100}{\text{mass of raw milk (kg)}} \times \frac{46}{\text{cheese moisture (g/100g)}} \quad (12)$$

In studies I and IV vat milk component recovery (CR) as the mass balance for each of the main milk components was calculated using equation (13).

$$CR\ (\%) = \left(\frac{\text{mass of cheese (kg)} \times \text{amount of component in cheese (g/100g)}}{\text{mass of milk (kg)} \times \text{amount of component in milk (g/100g)}} \right) \times 100 \quad (13)$$

Milk component recovery in the native whey (study II) and in the cheese whey (study V) was calculated using equations (14) and (15), respectively.

$$RY\ (\%) = \left[\frac{\text{mass in the MF permeate (g)}}{\text{mass in the skim milk (g)}} \right] \text{x } 100\ \% \quad (14)$$

$$RY\ (\%) = \left[\frac{\text{mass in the whey (g)}}{\text{mass in the cheese vat milk (g)}} \right] \text{x } 100\ \% \quad (15)$$

3.5.5 Functional property analysis of whey protein concentrate powders

The native whey and cheese whey protein concentrate (WPC) powder functional property analysis was performed in study VI. Solubility, viscosity, gelation, foaming, emulsifying capacity and water-holding capacity measurements were carried out.

3.5.5.1 Solubility

Solubility was determined according to IDF 173:1995 except that the pH of the protein dispersion (1%, w/v) was adjusted to 4.00 or 6.50±0.05 instead of 7.00±0.55 as described in the method.

3.5.5.2 Viscosity

Apparent viscosity of the WPC powders was measured using a Brookfield Model RVDVI+ viscometer (Brookfield Eng Labs Inc., Stoughton, MA, USA). Each powder was dissolved in deionised water (22°C) to make a 10% (w/w) protein solution. Viscosity was measured in triplicate using an ultra low adapter (Brookfield Engineering Labs Inc., Stoughton, MA, USA) and a spindle shear rate of 100 rpm.

3.5.5.3 Gelation

The strength of the gel was used as a measure of gelation as described by Rantamäki et al. (2000). The transparency and the appearance of the gels were also visually rated. A protein dispersion of 10% (w/v) was prepared with deionised water and its pH was adjusted to 6.5 with 0.1 M NaOH or 0.1 M HCl. The dispersion was stirred and further heated at 90°C for 10 min. After heating, the gel was cooled to room temperature. The penetration test was applied in a Lloyd Instruments Testing Machine (LR 10K, Lloyd Instruments, Fareham, England) using a 10 N tension load cell. The compression force at a depth of 5, 10, 15, 20, 25, 30, 35 and 40% of overall gel depth was recorded. For visual estimation the transparency of the gel was rated from 1 (transparent) to 5 (white). The appearance of gelation was rated from 0 (solution) to 5 (gel).

3.5.5.4 Foaming properties

Foam volume, overrun value and stability were determined as described earlier (Rantamäki et al. 2000). The used method was a modification of the methods described by Phillips et al. (1987) and de Wit et al. (1988b). Protein dispersion (3%, w/v) was whipped at maximum speed using a Hobart N-50 whipping machine (120 Watt, Hobart Canada, Ontario, Canada). The overrun was calculated as presented in equation (16).

$$\text{Overrun}\,(\%) = \left(\frac{\text{total volume of foam} \times \text{volume of dispersion}}{\text{volume of dispersion}} \right) \times 100 \qquad (16)$$

3.5.5.5 Emulsifying capacity

Emulsifying capacity (EC) indicates the maximum amount of oil which can be emulsified per unit weight of protein (Vuillemard et al. 1990). EC was measured according to the method of Vuillemard et al. (1990). Protein dispersions (pH 7.0) of 0.005-0.050% (w/v) were prepared. Protein solution (50 mL) was homogenized by an Ultra-Turrax T 25 (IKA-WERKE GmbH, Staufen, Germany) with rapeseed oil. EC was determined by observing the increase in electrical resistance at the inversion point. The EC value reported was the mean of 4 to 6 measurements.

3.5.5.6 Water-holding capacity

The water-holding capacity of WPC powders was measured according to the modified method of Quinn and Paton (1979) and Rantamäki et al. (2000). The volume of water needed to saturate the whey protein powder was determined. Samples were weighed in centrifuge tubes, a series of volumes of water was added and the mixtures were strongly agitated for 2 min. The samples were centrifuged at 20200 x g for 30 min at 10°C. The last sample was able to absorb all the water, whereas the first sample released some of the water. The mean of these two water volumes was taken as the water-holding capacity of the protein powder. Measurements were performed as triplicates.

3.5.6 Calculation of filtration parameters

In membrane filtration, important parameters are permeate flux, permeability of the desired components and the specific mass flux of separated components. In addition the energy consumption of separation processes was considered in these studies. Permeate flux (J) was calculated by diving permeate flow per hour (L/h) by filtration area (m^2) as described in equation 4 (Makardij et al., 1999). The specific mass flux of WP (M_{WP}) was calculated by multiplying flux (J) by the concentration of WP in the permeate ($C_{WP,\ permeate}$) (equation 17).

$$M_{WP} = J \cdot C_{WP,permeate} \qquad (17)$$

The specific energy consumption (E) for polymeric and ceramic membranes was obtained by dividing measured electric motor power consumption (Danfoss VLT HVAC Drive FC 100,

Graasten, Denmark; Vacon NXS FR4, Vaasa, Finland) by the specific mass flux of whey proteins (M_{WP}).

Permeation of whey proteins (P_{WP}) during microfiltration was calculated by dividing WP content in permeate ($C_{WP,\ permeate}$) by the WP content in retentate ($C_{WP,\ retentate}$) (equation 18).

$$P_{WP} = \frac{C_{WP,\ permeate}}{C_{WP,\ retentate}} \qquad (18)$$

Relative quantity (%) of α-LA ($Q_{\alpha\text{-}LA}$) was calculated by equation 19. Relative quantity (%) of β-LG ($Q_{\beta\text{-}LG}$) in skimmed milk and MF permeate was calculated as 100 - $Q_{\alpha\text{-}LA} = Q_{\beta\text{-}LG}$.

$$Q_{\alpha\text{-}LA} = \frac{C_{\alpha\text{-}LA}}{C_{\alpha-LA,} + C_{\beta-LG}} \text{ x } 100 \qquad (19)$$

where $C_{\alpha\text{-}LA}$ is the concentration of α-LA and $C_{\beta\text{-}LG}$ is the concentration of β-LG.

Permeation of β-lactoglobulin ($P_{\beta\text{-}LG}$) through hollow fiber (HF) membranes was calculated by dividing β-LG content in permeate (β-$LG_{,\ permeate}$) by β-LG content in retentate (β-$LG_{,\ retentate}$) (equation 20).

$$P_{\beta-LG} = \frac{C_{\beta\text{-}LG,\ permeate}}{C_{\beta-LG,\ retentate}} \qquad (20)$$

3.5.7 Statistical analyses

One way analysis of variance (ANOVA) and the statistical significance of the results from each trial were carried out using the Tukey HSD test with significance at $p<0.05$ using Statistica 7.1 (StatSoft. Inc., Tulsa, USA) software in studies I-VI. Shapiro-Wilk and Levene tests were used to measure normal distribution and equality of standard deviations of variables in study V.

4 RESULTS

4.1 Separation of whey proteins from skimmed milk with polymeric MF membranes

Polymeric microfiltration membranes were used to remove whey proteins from skimmed milk. The major whey proteins α-lactalbumin (α-LA) and β-lactoglobulin (β-LG) in the milk permeate were analyzed during the trials (Figure 11). Permeate whey protein concentration increased during the concentration phase (CF from 1 to about 4) and during the diafiltration phase (CF over 4) the protein concentration decreased linearly up to CF 61. α-LA and β-LG levels in skimmed milk were 0.11 and 0.30 % (w/w) and in the MF retentate of skimmed milk with CF 70 the levels were 0.01 and 0.03 % (w/w), respectively.

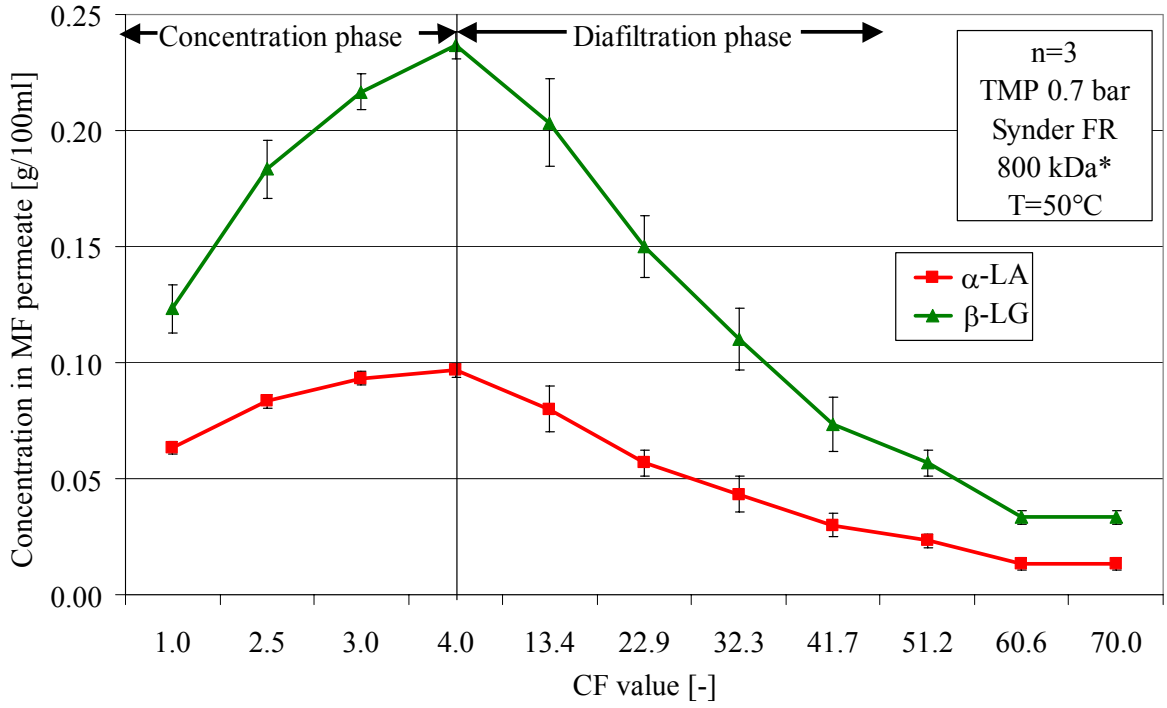

Figure 11. Permeation of α-LA (red) and β-LG (green) during skimmed milk microfiltration and diafiltration with spiral wound polymeric membrane (Synder FR, 800 kDa) at 50°C. CF=concentration factor, TMP=transmembrane pressure (bar), α-LA=α-lactalbumin, β-LG=β-lactoglobulin. *=membrane cut-off value.

Microfiltration permeate flux (J) is a result of membrane performance, which was mainly affected by membrane resistance (R_m), cake layer resistance (R_c), wall shear stress (τ_w), filtered liquid particle size and concentration (Vadi and Rizvi, 2001). Polymeric and ceramic MF membrane permeate fluxes of skimmed milk are presented in Figure 12. Polymeric membranes were able to filter skimmed milk at rather low flux values compared to ceramic

UTP-membranes. Mean permeate flux with the polymeric membrane was 13.4 L/m^{2}h (n=4) and with the ceramic membrane 73.9 L/m^{2}h (n=2) at a CF value of 1 to 4 at 50°C.

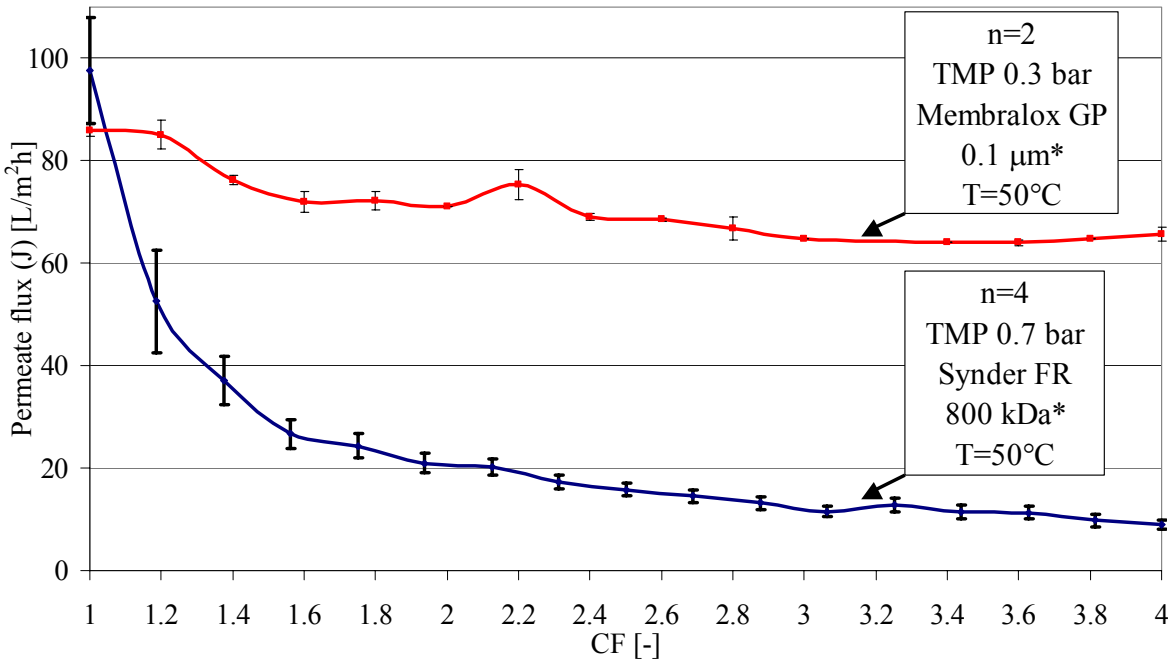

Figure 12. Skimmed milk microfiltration permeate flux (J) with polymeric (Synder FR, 800 kDa, blue) and ceramic (Membralox GP, 0.1 µm, red) MF membranes at CF values from 1 to 4 and at 50 °C. TMP=transmembrane pressure (bar), *=membrane pore size or cut-off value.

4.2 Effect of microfiltration parameters on permeate flux and β-lactoglobulin separation of skimmed milk

Permeate flux (J) and β-lactoglobulin (β-LG) mass flux in skimmed milk microfiltration at different tangential flow rates and transmembrane pressure values were measured with hollow fiber (HF) membranes using a CF value of 1.0. The permeability of β-LG with different filtration parameters was measured due to its greater effect on MF permeate total protein content. Increased tangential flow rates from 2.0 m/s to 3.5 m/s increased permeate flux values from 33 L/m^{2}h to 73 L/m^{2}h, respectively (Figure 13). Increase in TMP had a very limited effect on permeate flux values with HF membranes (data not shown). Mass flux of β-LG increased when higher tangential flow rates were used but the mass flux of β-LG was not increased at higher TMP values without increasing tangential flow rate (Figure 13). Mass flux of β-LG at 0.83 bar TMP varied from 60.0 to 113.6 g/m^{2}h when tangential flow rates were 2.0 and 3.5 m/s, respectively. A lower mass flux of β-LG was obtained due to lower permeation of whey proteins at higher TMP values. Permeation of β-LG varied from 71.9 to 49.2 % when TMP values were 0.36 (2.5 m/s) to 0.83 bar (3.5m/s), respectively. Increase of TMP from

0.49 bar to 0.83 bar with the same tangential flow rate of 2.5 m/s reduced β-LG permeation from 50.6 to 37.5 %, respectively. The sum of α-LA and β-LG mass flux varied between 77.8 and 144.8 g/m^{2}h when TMP values were 0.36 bar (2.5 m/s) to 0.83 bar (3.5m/s), respectively.

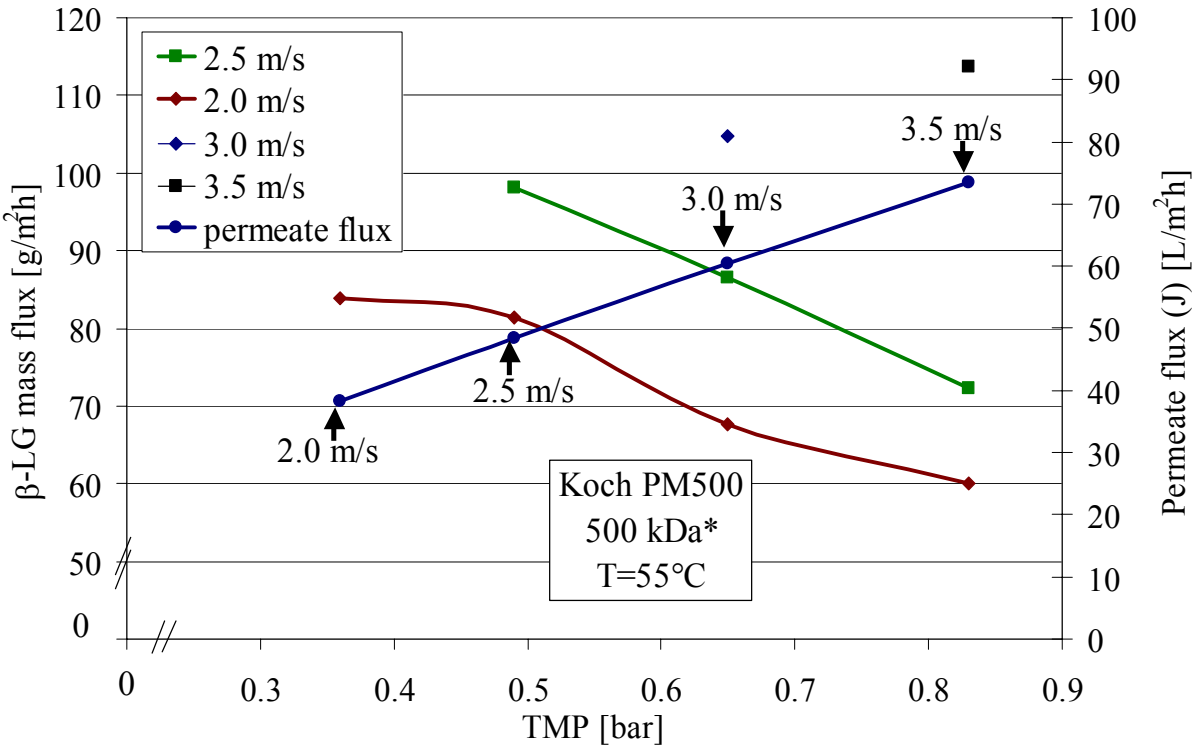

Figure 13. Skimmed milk microfiltration mass flux of β-lactoglobulin and permeate flux (J) with polymeric hollow fiber (Koch PM500, 500 kDa) membrane at transmembrane pressure (TMP) values of 0.36 to 0.83 bar and with tangential flow rates of 2.0 to 3.5 m/s. Red and green lines decribe β-LG mass flux values with tangential flow rates of 2.0 and 2.5 m/s, respectively, at different TMP values. The blue line describes permeate flux values with different TMP and tangential flow rate values. Concentration factor (CF) was 1. n=2. *=membrane cut-off value.

4.3 Comparison of ceramic and polymeric membranes in skimmed milk microfiltration

In milk microfiltration whey protein (WP) mass flux is actually a more important factor than permeate flux. Permeation of WP can be expressed as mass of protein divided by the energy consumption (P) which is needed for creating protein mass flux during filtration. The energy consumption (kW/kg of NWP) of polymeric (Synder FR, 800 kDa) and ceramic (Membralox GP, 0.1 μm) membranes in skimmed milk whey protein separation at 50°C is presented in Figure 14. The average energy consumption of the studied polymeric spiral wound (Synder FR, 800 kDa) MF membrane was 38.5% of the energy consumption of ceramic (Membralox GP, 0.1 μm) MF membrane. However, mean whey protein mass flux values with the polymeric and ceramic membranes were 41.2 g/m^{2}h and 305.4 g/m^{2}h, respectively. Due to

lower permeation and lower mass flux values of whey proteins, polymeric spiral wound MF membranes were a less interesting alternative when the performance of membrane area was calculated. However, polymeric MF membranes had lower energy consumption compared to ceramic MF membranes, due to lower tangential flow values (wall shear stress force (τ_w)) and more compact membrane packing (smaller spacer size of polymeric membranes compared to the hydraulic diameter of the filtration channel (d) of ceramic membranes). Ceramic membranes had ca. 7.4-fold higher mass flux values for whey proteins, but they also had ca. 2.6-fold higher energy consumption figures (Figure 14).

Permeation of whey proteins (P_{WP}) during microfiltration influences the WP content of skimmed milk MF retentate. The effect of lower permeation of polymeric membranes can be offset by using higher CF values or by using an additional diafiltration step. Mean permeation values of whey proteins (P_{WP}) with polymeric and ceramic membranes were 26.2% and 56.3%, respectively, during microfiltration (CF 1 to 4) at 50°C (Figure 14).

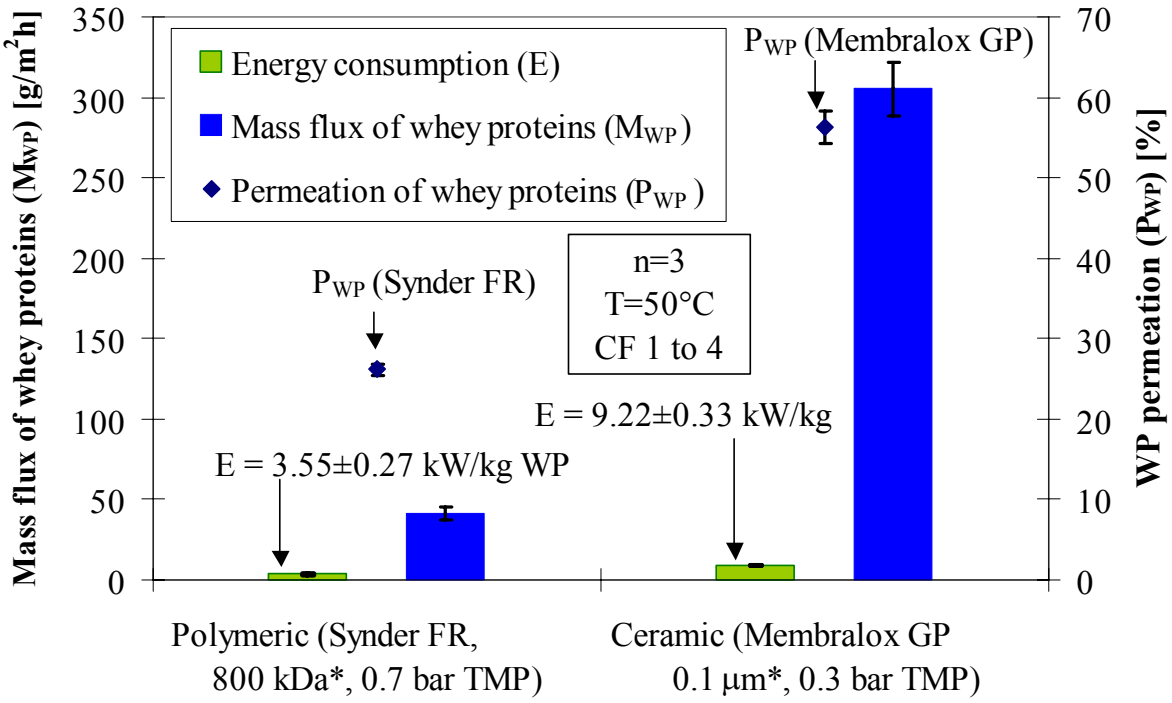

Figure 14. Energy consumption (E), whey proteins mass flux (M_{WP}) and whey protein permeation (P_{WP}) with the polymeric (Synder FR, 800 kDa) and ceramic (Membralox GP, 0.1 µm) microfiltration membranes in whey protein separation from skimmed milk (CF 1 to 4, n=3) at 50°C. CF=concentration factor, TMP=transmembrane pressure, *=membrane pore size or cut-off value.

Permeation of main whey proteins (β-LG and α-LA) was analysed in permeates from polymeric spiral wound and ceramic membranes and compared to the corresponding values

measured with skimmed milk. Relative quantities of $Q_{\alpha\text{-LA}}$ in both permeates (polymeric and ceramic) were higher than in skimmed milk and $Q_{\beta\text{-LG}}$ values were lower, as presented in Table 5.

Table 5. Mean relative quantities (α-LA+β-LG=100) of β-lactoglobulin ($Q_{\beta\text{-LG}}$) and α-lactalbumin ($Q_{\alpha\text{-LA}}$) in skimmed milk and permeates produced using polymeric membranes (Synder FR, 800 kDa) with a transmembrane pressure (TMP) of 0.7 bar and ceramic membranes (Membralox GP, 0.1 µm) with a TMP of 0.3 bar at 50ºC (CF 1 to 4).

	n	$Q_{\beta\text{-LG}}$ %	$Q_{\alpha\text{-LA}}$ %
Feed (Skimmed milk)	6	75.7±0.7	24.3±0.7
Permeate (Synder FR, 800 kDa)	3	73.3±2.0	26.7±2.0
Permeate (Membralox GP, 0.1 µm)	3	73.9±0.2	26.1±0.2

4.4 Cheese milk modification by micro- and ultrafiltration and its effect on Emmental cheese quality (I)

Milk microfiltration decreased native whey protein (NWP) / casein ratio as a function of CF value (study I), as seen in Figure 12. In Trial 1 (CF 1.0) the ratio was 0.19 and in Trial 5, in which intensive diafiltration was used, the ratio was 0.07. In all trials the β-casein / casein ratio was unchanged (0.41-0.42). Diafiltration with water reduced the milk ash content from 0.7% to 0.5%. Lower lactose and total solids contents in milk were measured in Trials 4 and 5 when diafiltration was employed (Figure 15).

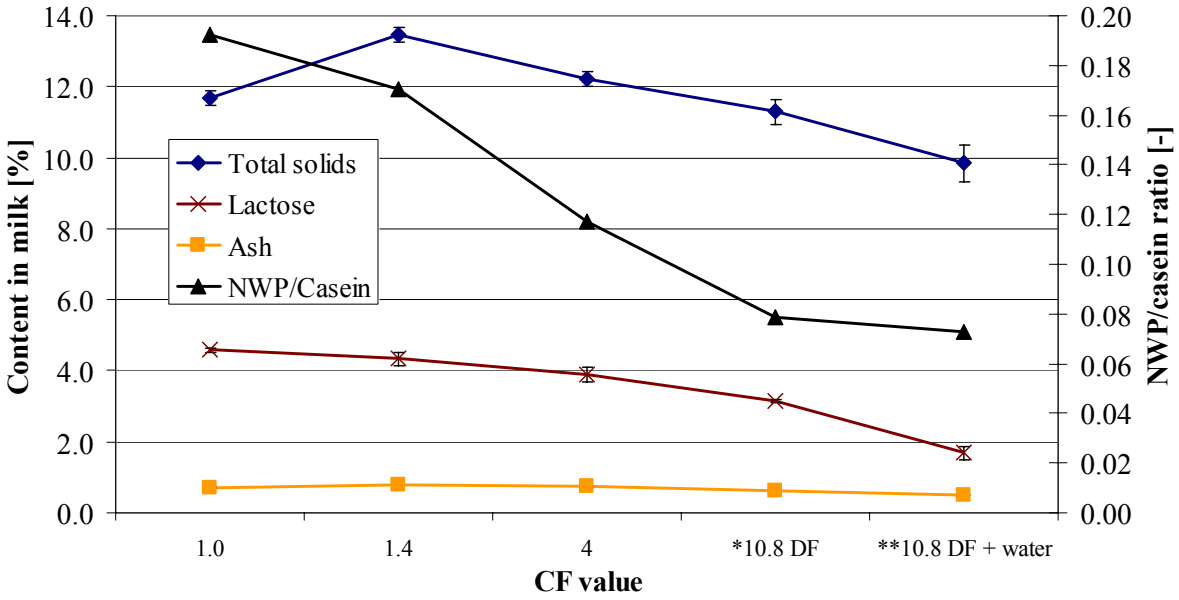

Figure 15. Effect of concentration factor (CF) value on total solids, total protein, lactose and NWP/casein ratio of cheese milk in study I. n=3, NWP=native whey protein. *=CF 10.8 including diafiltration with water, **=CF 10.8 including diafiltration with water and recombination of cheese milk with water.

Increased casein concentration in milk did not increase fat, casein or β-casein recovery in fresh cheese. Recovery of total solids increased and a significant increase in milk protein recovery was observed due to the elevated casein/TP ratio. Modification of milk composition had no effect on salt, moisture in the non fat substance (MNFS), fat on a dry basis (FDB), fat, casein or NWP contents of fresh cheese. Cheese yield was 8.5% with the reference milk and 11.2-11.8% with the modified milks. The adjusted cheese yields varied from reference cheese 8.6 to Trial 2 cheese 11.7%, which correlates with the lowest and highest milk casein contents. This result does not mean that microfiltration increases milk recovery yield to cheese, because the total amount of milk which was used for modified milks was not calculated.

In Trials 4 and 5 lower lactic acid content of fresh cheese, and lower acetic acid and propionic acid contents in ripened cheese were measured. pH values of milks varied from 6.5 to 6.8 and pH values of fresh cheeses from 5.2 to 5.75.

4.5 Influence of concentration factor on the composition of Emmental cheese milk and on the caseinomacropeptide content of the whey (II)

Total solids of native whey contained 7.5 to 10.0% (w/w) whey proteins, but for cheese whey the relation of TP in TS was 13.2% (study II). Casein permeation varied from 0.2 (CF 1.4) to 0.7% (CF 10.8) of the total amount of milk casein. The casein nitrogen/TN ratio increased from 78% (Trial 1, skimmed milk) to 92% when a CF value of 10.8 (Trial 5, cheese milk) was used. At a CF value of 1.4 NPN, lactose reduction was 29 and 24%. At a CF value of 10.8 the recovery yield of lactose in MF permeate was 91%.

In MF retentate at CF values of 4 and 10.8 a lower retention of α-LA (25.9 and 17.0%, respectively) compared to β-LG (44.0 and 25.1%, respectively) was observed (Figure 16.). Retentions of WPN in MF retentate were 95.5, 47.3 and 34.5% at CF values of 1.4, 4 and 10.8, respectively. At CF values 1.4 and 10.8, retention of milk protein in MF retentate was 98.5 and 78.4%, respectively.

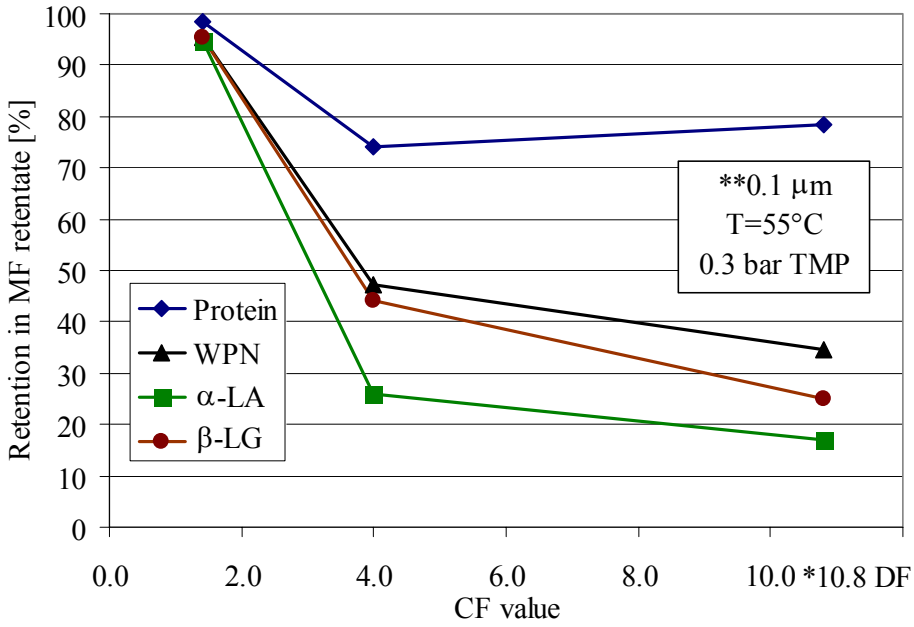

Figure 16. Influence of concentration factor (CF) value on protein, whey protein nitrogen (WPN), α-lactalbumin (α-LA) and β-lactoglobulin (β-LG) retention in skimmed milk retentate in study II. n=3. *=CF 10.8 including diafiltration step with water, **= microfiltration membrane pore size, TMP = transmembrane pressure during filtration, T=filtration temperature.

At a CF value of 1.4 the proportion of caseinomacropetides (CMP) in TP was below 20% and at CF 10.8 almost 40%. The relative amount of α-LA in WPN from cheese whey decreased from 12% to 5% when CF values of 1.4 and 10.8 were used, respectively. The relative amount of β-LG in WPN from cheese whey decreased from 40% to 20% when CF values of 1.4 and 10.8 were used, respectively. The recovery yield (RY) of total nitrogen (TN) from milk to whey was 21% in Trial 1 and 12% in Trial 5. TS recoveries from milk to whey were 47 and 26% in Trials 1 and 5, respectively. In cheese whey the CMP content varied from 4.3 to 5.5% according to the casein content of cheese milk. The TS content of cheese whey was 4.8% (w/v) in Trial 1 and 2.1% (w/v) in Trial 5.

4.6 Impact of milk modification on milk coagulation kinetics (III)

4.6.1 Composition of modified milks

Microfiltration of milk at a CF value of 1.4 resulted in higher total solids (TS) and native whey protein (NWP) contents of cheese milk. Microfiltration at CF values 4 and 10.8 NWP resulted in remarkably decreased lactose contents of milk, as shown in Table 6 (study III). The NWP/casein -ratio decreased in relation to CF value from 0.21 to 0.08 in milks 1 to 5,

respectively (Table 6, study III). Calcium content in milk was 1100-1400 mg/kg in all the studied milks. Milk diafiltration with water reduced the lactose content to 1.85% (w/v) in milk 5 with a TS content of 17%. In Trial 1 lactose contributed 36% of the TS content. In milks 2 to 5 a lower chymosin amount (0.083%) was used compared to milk 1 (0.100% chymosin) used as the reference milk. This was because preliminary results (data not shown) indicated much too rapid coagulation when the amount of chymosin added was the same as with reference milk. In test 2 the $CaCl_2$ addition was doubled, being 0.06% instead of 0.03% (tests 1 and 3).

Table 6. Total solids, total protein, native whey protein (NWP), NWP/casein ratio and lactose content of test milks in study III.

Content	Milk 1	Milk 2	Milk 3	Milk 4	Milk 5
Total solids [%]	12.5	14.3	12.4	12.4	10.9
Total protein [%]	3.6	4.52	3.38	4.42	4.32
Native whey protein (NWP) [%]	0.58	0.64	0.33	0.35	0.33
Native whey protein (NWP)/casein [-]	0.21	0.17	0.12	0.09	0.08
Lactose [%]	4.51	4.2	3.81	2.89	1.85

4.6.2 Coagulation results

Addition of $CaCl_2$ and decreased milk pH shortened the rennet clotting time (RCT) of milk and increased curd firmness (A40) in all the milks studied (study III), as seen in Figure 17. Curd firmness (A40) was 30% higher in milks 4 and 5 of trial 3. The time to reach K20 shortened from 21.7 min (milk 1) to 16.0 min and to 14.0 min in milks 4 and 5, respectively. RCT decreased from 15.0 min (milk 1) to 13.2 (milk 4) and 11.0 min (milk 5) in test 3. The amount of added chymosin was decreased for modified milks due the higher casein/TP ratio. The shortest RCT and the hardest curd firmness (A40 value) were obtained with milks 4 and 5, in which the casein/TP ratio was highest. Milks 4 and 5, in which the NWP content was lowest and the casein/TP ratio was highest, produced more than 50% lower K20-RCT time and more than 12% shorter RCT. Reduction of milk NWP/casein from 21% (milk 1) to 12% (milk 3) caused no changes in milk coagulation characteristics when the lower amount of chymosin (0.083%) was used (Figure 17). At higher casein levels the reduction of NWP amount resulted in lower RCT, K20 and K20-RCT values.

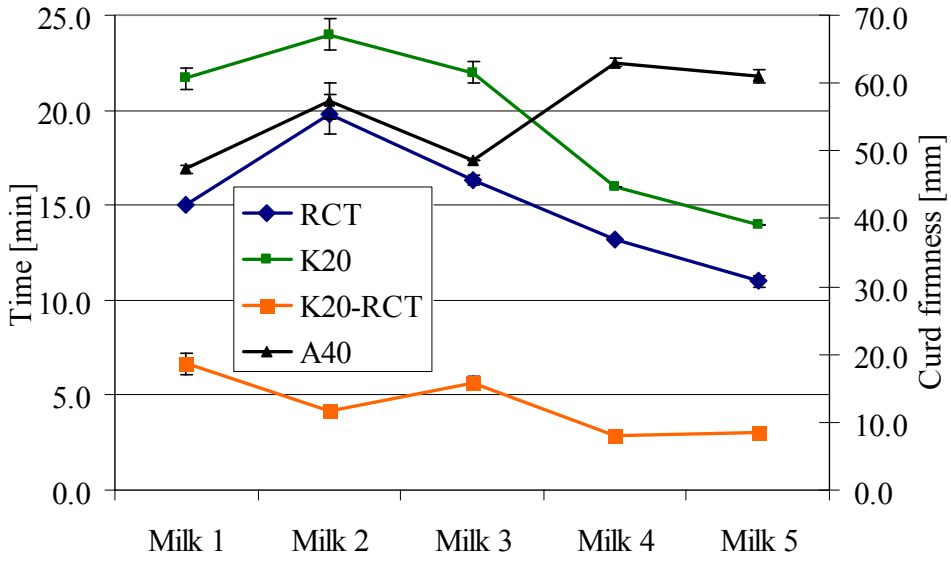

Figure 17. Modified milk coagulation properties in test 3 (pH adjusted to 6.50, $CaCl_2$ addition 0.03%) in study III. n=3. RCT=rennet clotting time is the time needed to detect gel formation; K20=time to reach a curd firmness of 20 mm, indicating optimal cutting time; A40=curd firmness 40 min after chymosin addition; K20-RCT=parameter which describes the rate of development of curd firmness. K20-RCT represents the time difference between clotting time and optimal cutting time for cheese manufacture; a shorter K20-RCT time means faster coagulation kinetics.

4.7 Pretreatment methods of Edam cheese milk. Effect on cheese yield and quality (IV)

4.7.1 Cheese milk composition

MF and UF cheese milks were standardized to have a TP of 4.2% and a fat/TP ratio of 0.7. In high temperature heat treated (HH) and reference (REF) milks the protein level was 3.4% and the fat/TP ratio was 0.7. In each vat the mass of total protein and casein varied from 16.5 (UF) to 17.0 kg (REF, HH) and from 13.4 (UF) to 13.9 kg (MF), respectively. Lactose contents in various milks varied from 4.3% (MF) to 4.7% (REF). However, in MF and UF milks mass reduction of total lactose was 25%, being proportional to the CF value. MF and UF increased the casein to whey protein (WP) ratio by 21% and 10%, respectively. HH increased the casein/WP ratio by 12%. The highest casein/α-LA and casein/β-LG ratios were 25.9 and 8.4, respectively, in MF milk and lowest ratios were 20.1 and 6.8, respectively, in the reference milk (REF). TS and fat contents in milks varied from 11.4 (HH) to 12.5% (UF) and from 2.40% (REF) to 2.95% (MF). In all milks the initial milk pH was 6.67 and during renneting the pH was 6.54 in MF milk and 6.59 in the reference milk.

4.7.2 Recovery of milk components in cheese and ripened cheese composition

Milk component recovery (CR) of TS in cheese was 55.5% for MF milk and 48.6 % for HH milk. Milk TP recovery in cheese was highest with MF milk (81.1%) and lowest with the reference milk (76.6%). Vat milk component recoveries (CR) of casein and fat varied from 92.1% (HH) to 93.8% (MF) and from 92.0% (REF) to 93.5% (MF and UF), respectively.

The cheese yield from vat milk (CY_v) was 12.8% cheese from milk with MF and UF, whereas with REF and HH the yield was 10.1% and 10.2%, respectively (Table 7). Adjusted cheese yield (ACY_r) from raw milk was 9.8% with MF, 10.4% with REF and 10.2% with HH and UF. In all milks the initial milk pH was 6.67. After the salting step the pH of cheese was 5.23 in UF cheese and 5.26 in REF and HH cheeses. Among ripened cheeses the MF cheese had the highest TP and the UF cheese had the lowest TP (Table 7). The highest fat content was in REF cheese 25.0% (w/w) and the lowest in the MF cheese 22.4% (w/w). In the MF cheese fat on a dry basis (FDB) was the lowest and in the REF cheese highest. The casein content was highest in the MF cheese (23.6% w/w) and the lowest in the REF cheese (21.8% w/w). Titratable fatty acid (TFA) contents varied between 50.8 and 75.8 mmol/kg in HH and REF cheeses, respectively (Table 7). Moisture of the non fat substance (MNFS) was highest in the UF cheese and lowest in MF cheese. Cheese fat content varied from 22.4% (w/w) MF to 25.0% (w/w) REF.

Table 7. The mean content of ripened cheese (w/w), cheese yield from vat milk (CY_v), cheese yield from ripened cheese (CY_r), moisture adjusted cheese yield from vat milk (ACY_v) and moisture adjusted cheese yield from ripened cheese (ACY_r) ± SD in study IV, (n=4).

	REF	**HH**	**MF**	**UF**
Fat [%]	25.0±0.5^{a}	23.7±0.5^{ab}	22.4±1.4^{b}	23.4±0.4^{ab}
Total solids [%]	55.9±0.9^{a}	54.9±0.7^{a}	54.4±0.8^{a}	54.3±0.5^{a}
Total protein [%]	26.0±0.5^{a}	26.1±0.4^{a}	26.6±1.0^{a}	25.5±0.5^{a}
Casein [%]	21.8±0.5^{a}	22.7±0.8^{ab}	23.6±0.9^{b}	22.2±0.3^{ab}
Salt [%]	1.5±0.1^{a}	1.5±0.1^{a}	1.5±0.1^{a}	1.5±0.1^{a}
MNFS [%]	58.8±0.9^{a}	59.2±0.6^{a}	58.9±0.7^{a}	59.7±0.5^{a}
FDB [%]	44.7±0.4^{a}	43.2±0.3^{ab}	41.2±2.1^{b}	43.2±0.5^{ab}
TFA (mmol/kg)	75.8±15.4^{a}	50.8±2.2^{b}	51.8±5.4^{b}	56.3±8.0^{b}
CY_v [%]	10.1±0.2^{a}	10.2±0.1^{a}	12.8±0.3^{b}	12.8±0.2^{b}
ACY_v [%]	10.4±0.1^{a}	10.2±0.2^{a}	12.7±0.5^{b}	12.6±0.2^{b}
CY_r [%]	10.1±0.2^{a}	10.2±0.1^{a}	9.9±0.3^{a}	10.3±0.2^{a}
ACY_r [%]	10.4±0.1^{a}	10.2±0.2^{ab}	9.8±0.3^{b}	10.2±0.2^{ab}

[a,b] Values within a row not sharing a common superscript differ significantly at $p<0.05$
REF=reference, HH=high temperature heat treatment, MF=microfiltration, UF=ultrafiltration, MNFS=moisture of the non fat substance, FDB=fat on a dry basis, TFA=titratable fatty acids.

4.7.3 Texture and sensory analysis of cheeses

The hardest cheeses were obtained with the HH milk and the softest with the REF milk. Hardness values of MF and UF cheeses were between those of REF and HH cheeses. The results of cheese cohesiveness were similar to the hardness results. Resilience of MF and UF cheeses was significantly higher than in REF and HH cheeses. No difference in springiness was detected between the cheeses. Results of cheese sensory analysis of the MF, UF and HH cheeses were comparable and the quality of these cheeses was regarded as excellent, but still the REF cheese was regarded as the softest. However, differences between the cheeses were negligible and statistically significant differences were not detected (Table 8).

Table 8. Sensory analysis of the Edam cheeses included in study IV.

Cheese milk	n	Appearance	Texture	Odour / taste	Total appearance
REF	17	9.08±0.75[a]	7.47±0.94 [a]	8.00±1.06 [a]	7.88±1.11 [a]
HH	14	8.50±1.02 [a]	8.21±1.19 [a]	8.36±1.01 [a]	8.36±0.93 [a]
MF	14	8.64±1.01 [a]	8.14±1.23 [a]	8.29±0.99 [a]	8.21±1.05 [a]
UF	14	8.79±0.80 [a]	8.21±1.48 [a]	8.36±1.08 [a]	8.36±1.22 [a]

[a] Values within a column not sharing a common superscript differ significantly at $p<0.05$
n = number of panellists in the sensory analysis
REF=reference, HH=high temperature heat treatment, MF=microfiltration, UF=ultrafiltration

4.8 Pretreatment methods of Edam cheese milk and their effect on the whey composition (V)

4.8.1 Composition of wheys and permeates

TS contents of MF and UF wheys were lower compared to REF and HH wheys (Table 9). TPs of MF and UF wheys were increased by about 8% compared to REF and HH wheys. The amount of WP was lower in MF whey (0.44%) than in UF whey (0.48%), but the lowest WP content was with HH whey (0.40%). In REF whey the WP content was 0.44%. The amount of casein was increased in MF and in UF wheys compared to REF and HH wheys. Lactose content was at approximately the same level in all the wheys studied. Non-glycosylated caseinomacropeptide (ngCMP) contents in MF and in UF wheys (0.08% and 0.07%, respectively) were higher compared to REF and HH wheys, which both contained about 0.06%. A similar level difference was also observed in the glycosylated caseinomacropeptide (GMP) content of the wheys, as can be seen from Table 9.

Table 9. The composition of unclarified reference whey (REF), high temperature heat treated whey (HH), microfiltration whey (MF) and ultrafiltration wheys (UF) in study V, mean±SD (n=4). (w/w).

	REF whey	**HH whey**	**MF whey**	**UF whey**
Total mass [kg]	518±5[a]	514±5[a]	403±3[b]	404±4[b]
TS [%]	5.23±0.13[a]	5.25±0.06[a]	5.23±0.05[a]	5.01±0.07[a]
Fat [%]	0.18±0.01[a]	0.19±0.02[a]	0.17±0.03[a]	0.20±0.04[a]
Lactose [%]	3.71±0.07[a]	3.82±0.04[a]	3.76±0.03[a]	3.66±0.08[a]
TP [%]	0.69±0.02[a]	0.69±0.01[a]	0.75±0.01[b]	0.77±0.01[c]
WP [%]	0.44±0.01[a]	0.40±0.01[b]	0.45±0.00[a]	0.48±0.01[c]
Casein [%]	0.06±0.01[a]	0.09±0.01[b]	0.095±0.00[b]	0.10±0.01[b]
NPN-P [%]	0.19±0.00[a]	0.20±0.01[a]	0.20±0.00[b]	0.19±0.01[a]
α-LA [%]	0.10±0.01[a]	0.09±0.01[a]	0.09±0.01[a]	0.09±0.01[a]
β-LG [%]	0.22±0.02[a]	0.21±0.01[b]	0.23±0.01[ab]	0.27±0.01[c]
GMP [%]	0.08±0.00[a]	0.10±0.01[b]	0.12±0.00[c]	0.11±0.00[d]
ngCMP [%]	0.06±0.00[a]	0.06±0.01[a]	0.08±0.00[b]	0.07±0.00[c]

[a,b,c,d] Samples sharing the same superscript are not statistically different (p>0.05), TS=total solids, TP=total protein, WP=whey protein, ngCMP=non-glycosylated caseinomacropeptide, GMP=glycosylated caseinomacropeptide.

Use of MF and UF as milk pretreatment methods resulted in 20% lower whey production compared to REF and HH processes (Table 10). The amount of total mass of TP was lower in MF and UF wheys (3.0 and 3.1 kg, respectively) compared to REF and HH wheys (3.6 and 3.5 kg, respectively). MF whey contained 20% less WP than REF whey (1.83 kg instead of 2.3 kg), but HH and UF wheys contained almost the same mass of WP (2.0 kg and 1.9 kg, respectively). Total mass of casein was highest in HH whey (0.45 kg) and lowest in REF whey (0.33 kg). Mass of fat in MF and UF wheys was 20% lower than in REF and HH wheys (0.8 kg instead of 1.0 kg).

MF and UF permeates of polymeric spiral wound membranes contained 23 and 25% of milk TS (6.0 and 4.9 kg, respectively), as can be seen from Table 10. Total masses of MF and UF whey were identical when the TP of milk was 4.2%. MF permeate contained 19% of milk NPN-P (0.2 kg) and 15% of WP (0.3 kg), respectively. UF permeate contained 4.9% of milk TP (0.2 kg), which consisted totally of NPN-P. MF permeate contained low amounts of high molecular mass whey proteins such as BSA, lactoferrin (LF), or immunoglobulin G (IgG), but did contain traces of casein (Table 10). The total amount of casein in the MF permeate was 0.04 kg, corresponding to 0.25% of the vat milk casein (about 400 kg milk containing a total amount of casein of 16.8 kg, Table 10). Total masses of ngCMP (0.3 kg) and GMP (0.4-0.5 kg) were at the same level in all wheys (Table 10), being 0.06-0.12% of the total mass of whey. The total mass of caseinomacropeptides varied between 720 and 810 g.

Table 10. Mass of initial vat milks and the mass balance [kg] of unclarified reference whey (REF), high temperature heat treated whey (HH), microfiltration whey (MF), ultrafiltration whey (UF), MF permeate and UF permeate components in study V, n=4. Higher mass of whey than of milk was the result of water addition during the cheese cooking phase.

	REF milk	**HH milk**	**MF milk**	**UF milk**		
Total mass [kg]	500±2	499±3	400±1	402±4		
	REF whey	**HH whey**	**MF whey**	**UF whey**	**MF permeate**	**UF permeate**
Total mass [kg]	518±5^{a}	514±5^{a}	403±3^{b}	404±4^{b}	116±8	95±4
TS [kg]	27.0±0.5^{a}	27.0±0.3^{a}	21.1±0.2^{b}	20.2±0.1^{b}	6.0±0.2	4.9±0.4
Fat [kg]	0.96±0.02^{a}	0.98±0.01^{a}	0.77±0.06^{b}	0.76±0.03^{b}	0	0
Lactose [kg]	19.2±0.2^{a}	19.7±0.3^{a}	15.1±0.1^{b}	14.8±0.2^{b}	5.1±0.2	4.3±0.4
TP [kg]	3.58±0.24^{a}	3.53±0.04^{a}	3.03±0.03^{b}	3.10±0.02^{b}	0.56±0.02	0.17±0.02
WP [kg]	2.29±0.02^{a}	2.03±0.04^{b}	1.83±0.01^{c}	1.94±0.02^{d}	0.34±0.01	<0.01
Casein [kg]	0.33±0.03^{a}	0.45±0.03^{b}	0.38±0.02^{a}	0.40±0.04^{b}	0. 04±0.00	0.00±0.00
NPN-P [kg]	0.97±0.01^{a}	1.03±0.05^{a}	0.82±0.01^{b}	0.77±0.01^{b}	0.19±0.05	0.16±0.02
α-LA [kg]	0.50±0.03^{a}	0.44±0.03^{b}	0.35±0.02^{c}	0.36±0.03^{c}	0.07±0.00	0
β-LG [kg]	1.21±0.07^{a}	1.09±0.04^{b}	0.91±0.02^{c}	1.07±0.05^{b}	0.20±0.01	0
GMP [kg]	0.41±0.00^{a}	0.51±0.04^{bc}	0.48±0.00^{cd}	0.44±0.01^{ad}	0	0
ngCMP [kg]	0.31±0.00^{a}	0.28±0.03^{b}	0.32±0.00^{a}	0.28±0.00^{b}	0	0

^{a,b,c,d} Samples sharing the same superscript are not statistically different (p>0.05), statistical analysis was carried out only between cheese wheys. TS=total solids, WP=whey protein, NPN-P= non-protein nitrogen converted to protein equivalent by multiplying by 6.38, ngCMP=non-glycosylated caseinomacropeptide, GMP=glycosylated caseinomacropeptide.

The RY of vat milk TS in whey was lowest for UF (40.5%) milk and highest for HH (47.6%) milk. RYs of fat in whey were 6.5% for MF and UF milks, but 8.0% and 7.5% for REF and HH wheys, respectively. RY of WP was lowest for UF whey (85.4%) and highest for REF whey (89.3%). RYs of the major whey proteins α-LA and β-LG were lowest for UF whey (60 and 57%, respectively) and highest for REF whey (77 and 63%, respectively). RY of CMP in whey varied between 2.1% (HH and UF) and 2.3% (REF and MF).

4.8.2 WPC powders

SDS-PAGE results showed that all WPC powders (study V) contained BSA, LF and IgG, but this method was unable to quantify these proteins. For further comparison of these WPC powders, their amino acid composition was analyzed (Figure 18). The most important amino acids for this study were threonine (Thr) and tryptophan (Trp), due to the importance of these amino acids for infant nutrition. Whey protein products are commonly used in the infant nutrition industry. MF WPC and REF WPC contained Thr at 7.1% and 6.8% of total amino acids, respectively. NWPC contained Thr 4.7% of total amino acids. Trp contents varied from 1.6% (MF WPC) to 1.8% (REF WPC) of total amino acids. The content of Trp, 2.2%, was the highest in NWPC of total amino acids. The greatest difference was also between the traditional WPC powders and the NWPC powder in the case of many other essential amino

acids (Figure 18). NWPC contained higher amounts of Leu, Lys and Trp due reason that NWPC contained no CMP which is rich of aromatic amino acids like Trp, Phe and Tyr.

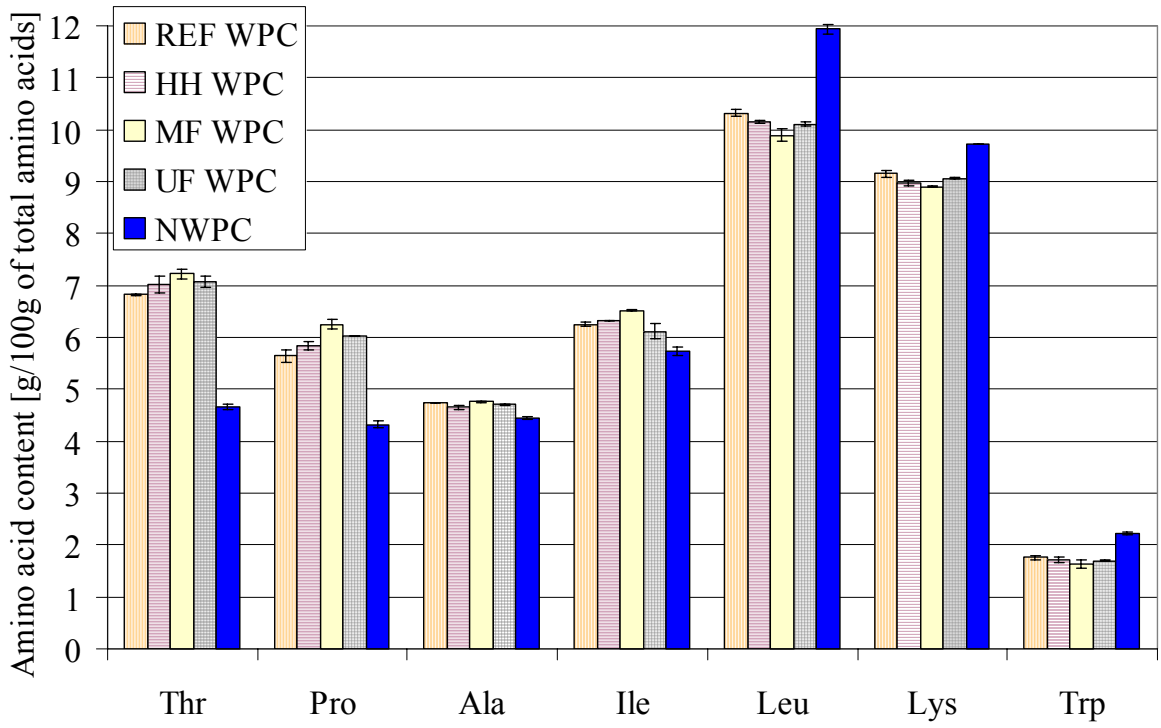

Figure 18. Essential amino acid composition [g/100g of total amino acids] of whey protein concentrates (WPC) made from untreated reference (REF WPC), high temperature heat treated (HH WPC), microfiltrated (MF WPC) and ultrafiltrated (UF WPC) wheys in study V. Native whey protein concentrate (NWPC) is presented as a reference. Mean±SD (n=2). Only those amino acids of which the content in WPC powders showed statistically significant differences ($p<0.05$) are presented. Amino acids Thr=threonine, Pro=proline, Ala=alanine, Ile=isoleucine, Leu=leucine, Lys=lysine, Trp=tryptophan.

4.9 Functional properties of whey protein concentrate powders (VI)

4.9.1 Composition of WPC powders

Powders of native whey protein concentrate (NWPC-SD and NWPC-FD) had lower protein and fat contents compared to powders of cheese whey protein concentrate (CWPC-FD and WPC-SD). In this study the CMP content of CWPC total protein content was 14-17%, whereas NWPC powders did not contain any detectable CMP.

4.9.2 Functional properties of WPC powders

NWPC powders had significantly higher solubility at pH 4.0 than at pH 6.5. No differences in solubility between freeze dried or spray dried NWPC powder were detected. Similar results were also obtained by Vaghela and Kilara (1996) and Bhargava and Jelen (1995). WPC powder made from cheese whey had lower solubility than NWPC powders and industrial spray dried WPC had the lowest solubility values. Differences in solubility between NWPC and CWPC powders were reported by Britten and Pouliot (1996), supporting the results obtained in this study (VI).

Increasing concentration increases the viscosity of an aqueous whey protein solution (Kessler, 2002a). In this study (VI) viscosity was measured at a protein content of 10% (w/v), but no significant differences in viscosity between NWPC and CWPC powders were detected. NWPC-SD had the highest viscosity and NWPC-FD the lowest viscosity at the same protein level, but comparison of these results was difficult due to variation in the contents of lactose and minerals between the samples. Analogous results for the viscosity of WPC powder solutions were also obtained by Moon and Mangino (2004).

Significantly higher gel strength values were obtained for NWPC powders compared to CWPC powders (Figure 19). NWPC-FD and NWPC-SD had a more compact gel structure, which was easy to cut without loss of water. WPC-SD gave an elastic gel which differed remarkably from the gel obtained for CWPC-FD.

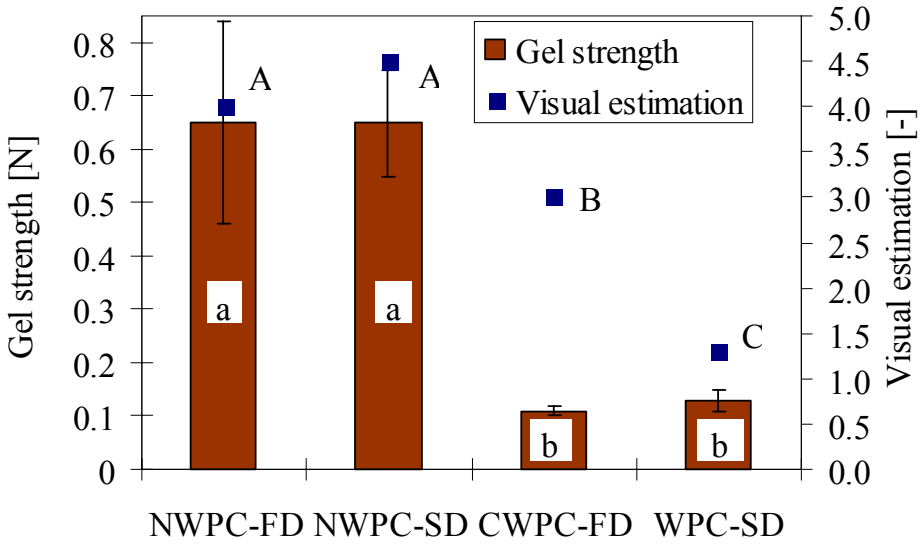

Figure 19. Gel strength and gel visual estimation (0 = solution or precipitation, 5 = elastic gel) of 10% (w/v) protein dispersion made of freeze dried native whey protein concentrate (NWPC-FD), spray dried native whey protein concentrate (NWPC-SD), freeze dried cheese whey protein concentrate (CWPC-FD) and industrial spray dried cheese whey protein concentrate (WPC-SD) powders at 90°C for 10 min in study VI. n=6. Means with different letters, a-b and A-C, are significantly different ($p<0.05$).

NWPC powders had excellent foaming properties compared to CWPC powders. Foam volume was over sixfold and overrun was over fivefold higher with NWPC powders compared to CWPC powders. In addition, foam stability was much better with NWPC powders. Foam overrun and volume with NWPC powders were higher compared to egg white, but foam stability was at about the same level. Emulsification capacity (EC) was statistically ($p<0.05$) higher with NWPC powders at protein concentrations of 0.0125 and 0.050% (w/v). Water-holding capacity of NWPC powders and CWPC-SD were similar, but industrial WPC-SD had higher water-holding capacity although lower solubility.

5 DISCUSSION

5.1 Separation of whey proteins from milk with polymeric MF membranes

During milk microfiltration whey proteins pass partially through the membrane, depending on membrane resistance and filtration conditions. The main whey proteins in milk permeate, α-lactalbumin (α-LA) and β-lactoglobulin (β-LG), were analyzed during trials in this study. Summarized results for whey protein content as a function of the concentration factor (CF) value are shown in Figure 11. To obtain the same filtration permeate flux with polymeric membranes as with ceramic membranes, the total membrane area should be 5.5 times higher (Figure 11). This is particularly evident if higher CF values (>3.0) are used. The reason for the lower flux rate at higher CF values is the increased viscosity of skimmed milk retentate (Vadi and Rizvi, 2001) and denser and thicker casein gel layer on the membrane surface (Jimenez-Lopez et al., 2008).

As expected, whey protein content in permeate increased during the concentration phase and protein content during the diafiltration phase decreased linearly up to CF 61. Kulozik and Kersten (2002) reported an increase of the casein/whey protein -ratio as a function of diafiltration steps when using ceramic uniform transmembrane pressure (UTP) membranes. The shape of the whey protein (WP) permeation curve is related to the membrane characteristics. A membrane with high WP permeation rate increases the WP content in retentate during the concentration phase less and the decrease in WP content in the retentate during diafiltration is faster.

Permeate flux (J) in skimmed milk microfiltration is a function of membrane performance under the filtration conditions. Compared to ceramic UTP-membranes, polymeric membranes are able to filtrate at rather low flux values, reported by Lawrence et al., 2006. Permeate fluxes of polymeric and ceramic MF membranes are presented in Figure 12. Flux rate decreased rapidly when the CF value increased, due to increase in retentate viscosity, concentration polarisation and membrane fouling as was reported also by Kulozik and Kersten (2002). Permeate flux reduction with a ceramic membrane was moderate, due to higher tangential flow rates on the membrane surface. Spiral wound MF membranes are not used in high tangential flow conditions, because membrane spacers between membrane leaflets are damaged if pressure drop (related to tangential flow) over the membrane increases

above 0.9 bar. In addition, transmembrane pressure (TMP) values were higher with polymeric membranes due to operation in non-uniform transmembrane pressure mode, where the risk of centrifugal pump cavitation cannot be avoided when the permeate side is also pressurized. Therefore similar TMP values for polymeric spiral wound and ceramic membranes cannot be used.

Zulewska et al. (2009) reported that ceramic membranes are able to remove whey proteins with less membrane area compared to polymeric membranes and diafiltration is necessary only for polymeric membranes. However, any energetic calculations of whey protein removal with ceramic and polymeric membranes were not presented which is the most important factor for practical applications.

5.2 Effect of microfiltration parameters on permeate flux and β-lactoglobulin separation of skimmed milk

The tangential flow on a membrane surface creates wall shear stress (τ_w) force, which reduces the height and compactness of the cake layer (Zeman and Zydney, 1996) and affects total filtration resistance (Vadi and Rizvi, 2001). Hollow fiber (HF) results (Figure 13) showed that increased tangential flow rate resulted in a linear increase in permeate flux values in skimmed milk microfiltration and results were in line with previous studies (Gésan-Guiziou et al., 1999b; Gésan-Guiziou, et al., 2000). Permeate flux of HF membranes was higher compared to polymeric spiral wound (SW) microfiltration membranes but lower than with ceramic membranes. This was as expected, due to the tangential flow rates which were lowest with SW membranes and highest with ceramic membranes. However, increased TMP values had only a limited effect on permeate flux values with HF membranes and therefore it can be concluded that in skimmed milk microfiltration the cake (casein gel) layer thickness and compactness were also limiting permeate flux values in the filtration conditions used, as concluded also Piry et al. (2008).

Tangential flow rates and transmembrane pressure (TMP) values had a major impact on mass flux of β-lactoglobulin (β-LG). At lower tangential flow rates (2.0 m/s), lower mass flux values of β-LG were attained compared to the highest (3.5 m/s) tangential flow rates, as expected, but the reduction of mass flow rate with increased TMP values proved that cake layer compactness has a great effect on mass flux of β-LG. When the permeate flux was not

decreasing the reason for lower mass flux of β-LG was decrease in membrane permeability of β-LG. HF results showed that permeation can vary remarkably with the same membrane and different filtration parameters (Figure 13). It can be concluded that the permeate flux was a less important factor in skimmed milk microfiltration than mass flux of whey proteins (here β-LG), and that increased TMP value had a negative influence on permeability of β-LG. When mass flux values of whey proteins (α-LA and β-LG) through hollow fibre, polymeric spiral wound (SW) and ceramic membranes were compared it was observed that the lowest mass flux of WP was obtained with polymeric SW membranes and the highest with ceramic membranes (Figures 13 and 14). Different tangential flow rates and overall membrane resistances explained the differences in mass flux values between SW and ceramic membranes, which were also reported by Gésan-Guiziou et al. (1999b) in a study of ceramic membranes in different filtration conditions.

5.3 Comparison of ceramic and polymeric membranes in skimmed milk microfiltration

In milk, microfiltration permeate flux (J) is less important than whey protein (WP) mass flux (M_{WP}) reported Piry et al. (2008). Permeation of WP can be expressed as mass of protein divided by the energy consumption (kW/kg of WP) during filtration which is needed to create WP mass flux. In milk microfiltration tangential flow with ceramic membranes is often higher than 7 m/s (Saboya and Maubois, 2000) and with polymeric spiral wound (SW) membranes much lower, 0.5 to 3.0 m/s. However, high tangential flow rates with polymeric spiral wound (SW) membranes could not be estimated due to the membrane spacer structure, and therefore wall shear stress values would be a better parameter to characterize friction force on the SW membrane surface (Schwinge et al., 2002). Ceramic membranes operate in the range where fluid hydrodynamics largely account for permeate formation (Altmann and Ripperger, 1997). Permeate flux of polymeric SW membranes is controlled by back-diffusion due to low tangential flow rates (Guillen and Hoek, 2009), which also caused lower energy consumption per kg of WP. This causes almost tenfold lower whey protein mass flux (M_{WP}) values with polymeric SW membranes, but because of much more effective membrane packing the polymeric membranes are more effective than ceramic membranes in the separation of whey proteins from skimmed milk if performance is measured as floor area (kg of WP/ m^2) which is needed for filtration equipment to separate the same amount of whey protein. Polymeric membranes had a lower permeation rate of whey proteins (P_{WP}) compared with ceramic membranes, as can be seen from Figure 14. In a recent study Zulewska et al. (2009) also

reported higher WP retentions and lower permeate flux values with polymeric spiral wound (SW) microfiltration membranes compared with ceramic UTP and gradient permeability (GP) membranes. In this study measurement of hollow fiber membrane energy consumption was not performed and therefore comparison with other membranes could not be made.

The relative amounts of β-LG ($Q_{\beta\text{-LG}}$) and α-LA ($Q_{\alpha\text{-LA}}$) in microfiltration permeate suggested that permeation of α-LA was higher compared to the values measured for skimmed milk. However, no statistical differences between polymeric and ceramic membranes were detected, although a slightly higher relative amount of β-LG was observed with ceramic membranes, as recently reported by Zulewska et al. (2009). However, they had no explanation for that kind of phenomena. It could be assumed that lower permeability of β-LG from polymeric membranes is dependent of membrane concentration polarization layer thickness and density when β-LG is larger molecule (dimer) at milk natural pH than α-LA. This may cause decreased β-LG flow through polarization layer and membrane itself so resistance for β-LG through membrane is higher than for α-LA. However, Tolkach and Kulozik (2006) reported that permeability of β-LG is complicated phenomena and it can`t be only explained with interactions between membrane, deposit layer and β-LG.

Membrane performance depends on many factors such as spiral wound (SW) membrane configuration (Bégoin et al., 2006), channel height, and shape and length of ceramic membranes (Grangeon and Lescoche, 2000). Energy consumption during filtration also depends on pump type, type of electric motor and filtration unit structure. In this study polymeric SW and ceramic membranes were used in the same process, enabling direct comparison of these membranes. The mean energy consumption of a polymeric SW membrane was considerably lower compared to the energy consumption of ceramic membrane, resulting in lower running costs of filtration. Schier and Paar (2007) compared the behaviour of polymeric and polymeric membranes in milk microfiltration, but no efficiency calculations were included. Energy efficiency is a critical factor in milk microfiltration due to the high volumes, high CF values and relatively low product prices. Ceramic membranes of skimmed milk microfiltration membranes can tolerate high tangential flow rates of >8 m/s in order to obtain low retention of whey proteins and high permeate flux values (Samuelsson et al., 1997b). High wall shear stress (τ_w) values were not used with polymeric membranes due to their poorer mechanical strength, which lowers the critical flux value. The critical value of J/τ_w is therefore lower and this relation characterizes competition between erosion and

convection at the solution/membrane interface, as was reported by Le Berre and Daufin (1996). Mass transport of skimmed milk microfiltration is dependent on particle (casein micelle) size, interactions between particles (casein micelles and whey proteins) and interactions between particles (casein micelles) and membrane (Ripperger and Altmann, 2002). Based on mass transport theory, whey protein separation and permeate flux with polymeric membranes are therefore more related to diffusion than erosion forces. Separation of whey proteins and high permeate flux with ceramic membranes were achieved using hydrodynamic forces (wall shear stress (τ_w)), which reduced the thickness of the casein gel layer and allowed higher whey protein mass flux and permeate flux values.

In skimmed milk microfiltration, membrane performance has a great influence on the physical size of filtration equipment and therefore also on filtration costs. Low permeability of whey proteins and low permeate flux lead to high investment and running costs. Zulewska et al. (2009) reported that ceramic membranes are able to separate same mass of whey proteins on one stage system and polymeric systems are needed three stage with diafiltration to do same separation work. However, it must be understood that high permeate mass flux of whey proteins is not sufficient if membrane price and energy consumption of the installation are very high, as is usually the case with ceramic membranes. Polymeric SW membranes are typically inexpensive (20-70 \$/m^2), hollow fiber membranes are expensive (>1 700 \$/m^2) and ceramic membranes are very expensive (>10 000 \$/m^2) (Wagner, 2001). This means that polymeric SW membrane filtration installations with satisfactory whey protein mass flux and permeate flux values can be more economical than ceramic membrane installations.

5.4 Cheese milk modification by micro- and ultrafiltration and its effect on Emmental cheese quality (I)

The effects of composition of modified milk on Emmental cheese yield, quality and ripening were studied. In this study MF and UF techniques were used to remove whey proteins and to reduce the milk lactose and mineral content. At the same time the influence of increased milk casein level on the recovery of milk components was studied. There was no previous report on the minimum lactose content of Emmental cheese vat milk required for sufficient lactic acid production to decrease the cheese pH to 5.2. Lactose reduction before milk renneting can decrease or eliminate the need for addition of dilution water, and minimizes the amount of sweet cheese whey obtained.

The recovery of total solids from milk to cheese naturally increased when the lactose and mineral contents of cheese milk were reduced. Neocleous et al. (2002a) reported that an increase in milk casein concentration can increase casein recovery, but in our study (I) this effect was not observed. When the amount of native whey proteins (NWP) in milk was reduced the recovery of residual NWP in cheese increased. One reason for this could be the firmer structure of cheese coagulum, which trapped NWP during syneresis (Garem et al., 2000). As expected, a statistically significant increase in milk protein recovery was observed when an increased amount of NWP was removed from milk, producing an increase in the casein/TP ratio. Modification of milk composition had no influence on the contents of salt, moisture in the non fat substance (MNFS), fat on a dry basis (FDB), fat, casein and NWP in fresh cheese.

For the ripening of Emmental cheese it is important to reduce the cheese pH to 5.2. Reduced lactose content of cheese milk resulted in reduction of lactic acid production, which caused decreased acetic acid and propionic acid levels in ripened cheese. Typical flavour of Emmental cheese was not achieved if the milk lactose content was reduced by more than 25% (w/w). Larsson et al. (2006) and Upreti et al. (2006) reported that lower lactose, salt (Ca and P) and NWP contents increased the rate of proteolysis. In this study (I), decreasing the β-casein/TS ratio in ripened cheese promoted increased proteolysis. One reason for the higher rate of proteolysis was probably the higher pH of ripened cheese, resulting in higher activity of plasmin. In modified milks native whey protein (NWP), which could operate as plasmin inhibitors, were partially removed as reported Benfeldt, 2006. In this study, it was concluded that the lactose content of Emmental cheese milk should be 3.2 to 3.9% in order to reach the desired acetic and propionic acid levels in ripened cheese and to reach a pH value of 5.2 in the fresh cheese.

5.5 Influence of concentration factor on the composition of Emmental cheese milk and on the caseinomacropeptide content of whey (II)

The amount and quality of cheese whey were affected by MF/UF treatment of cheese milk. In this study (II) one aim was to characterize the effects of cheese milk composition and increased milk protein content on the quality of whey. In milk microfiltration, native whey was formed, containing native whey proteins at a level depending on the milk CF value applied, but lacking caseinomacropeptides (CMP). This kind of pretreatment of milk caused changes in the amount and composition of cheese whey.

As expected, retention of casein in skimmed milk microfiltration (MF) was very high, but retention of whey proteins in MF milk caused an increased native whey protein nitrogen (WPN) content in milk retentate, and in turn reduced the content of WPN in MF permeate. Lower WPN content in MF permeate was partially caused by absence of CMP, which normally represents 15-25% of TP in cheese whey (Thomä-Worringer et al, 2006). However, intensive microfiltration increased the casein content from 78% of total nitrogen in skimmed milk to 92% of total nitrogen at a CF value of 10.8 of microfiltered milk. A significantly higher α-LA/β-LG ratio was observed in MF permeate compared to skimmed milk, as was also reported by Tolkach and Kulozik (2005). Lower permeability of β-LG was due to higher molecular mass, effective hydrodynamic size and complicated interactions between membrane, deposit layer and β-LG. Removal of NPN and lactose was efficient already at a CF value of 1.4. At a CF value of 10.8 the reduction of lactose content increased the total protein (TP) / total solids (TS) ratio.

During milk coagulation CMPs are released from κ-casein in the casein micelles by the action of chymosin, and end up in the whey. When cheese milk contained less whey protein nitrogen (WPN), e.g. at CF 10.8, a lower recovery yield of WPN in cheese was obtained. Higher CF values resulted in an increase in CMP and decreases in α-LA and β-LG of WPN in cheese whey, which was natural due to the lower α-LA and β-LG contents in cheese milk. Lower lactose level in milk caused an increased TP/TS ratio in cheese whey, but no influence on the ash/TS ratio was observed. Modification of milk composition did not influence CMP formation during milk coagulation, as has been reported Swaisgood (2003). This might be because the casein content was not increased in cheese whey, which could affect CMP formation.

5.6 Impact of milk modification on milk coagulation kinetics (III)

Milk modification causes changes in milk coagulation properties, which were already observed in study II. These changes were further analysed in study III. The NWP/TP -ratio in vat milk decreased in relation to cocentration factor (CF) value. Calcium content in milk was not dependent on the CF value, due to the high proportion of calcium attached to casein micelles. Milk diafiltration with water increased milk pH, causing increased rennet clotting time (RCT).

The factors affecting milk coagulation properties have been widely studied (McMahon and Brown, 1982; Famelart et al., 1996; Steffl et al., 1996; Caron et al., 1997; Famelart et al., 1999; Ng-Kwai-Hang et al., 2002). Addition of $CaCl_2$ and lowering milk pH shorten the milk rennet clotting time (RCT) and increase curd firmness (A40) as was also reported Nájera et al. (2003). It has been possible to reduce the amount of chymosin added to modified milks due to their higher casein/TP ratio. One reason for shorter RCT might have been the lower NaCl content in modified milks (Karlsson, 2006), due to diafiltration with water. However, the shortest RCT and hardest curd firmness was obtained with milks in which the casein/TP ratios were the highest. This relation was also has described by Daviau et al. (2000b). McMahon et al. (1993) claimed that in concentrated milks a lower level of κ-casein hydrolysis promotes gel network and reduces RCT in this way. Decrease of milk pH to 6.5 increased the coagulation rate more in the cases of 4 and 5 in study IV, where the initial pH of milk was elevated due to diafiltration with water. Milks 4 and 5 had the lowest NWP content and the highest casein/TP ratio, which could explain the increased K20-RCT and reduced RCT values. A similar observation was also made by Garem et al. (2000). The amount of rennet was reduced by 20% with the modified milks. Despite this, milk rennetability was enhanced especially with milks 4 and 5, in which NWP and lactose contents were the lowest. This indicates that dosage of rennet added could be reduced by more than 20% without affecting the coagulation properties. It has been reported that UF milks coagulate faster and that the curds produced are more rigid (Casiraghi et al., 1988; Sharma et al., 1992), but in the case of MF the coagulation rate is enhanced even more, as reported by Schreiber et al. (2000). Reduction of milk NWP/TP ratio from 16.1% (milk 1) to 9.8% (milk 3) caused no changes in milk coagulation characteristics when 20% less rennet was used. In the same study (IV) it was observed that the lactose/TS ratio, which decreased from 36% (milk 1) to 31% (milk 3), can influence milk coagulation. At higher casein levels reduction of NWP resulted in reduced RCT, K20 and K20-RCT values. In the literature, whey proteins (Caron et al., 1997) and salts (Tsioulpas et al., 2007) have been reported to impair chymosin activity as was seen also in this study. Firmer coagulation is evidently caused by lower amounts of filling material (NWP, lactose) in casein matrix, as proposed Mahaut and Korolczuck (1992). This filling material slows down rennet diffusion due to lower milk viscosity, and reduces rennet activity. The increased casein/TP -ratio enhances the coagulation rate. Increased casein/TP -ratios in cheese manufacture necessitate a more precise process control and different process parameters such as reduced cutting temperature, reduced amount of rennet or more precise cutting time.

However, a reduced amount of chymosin can slow down cheese ripening (Benfeldt, 2006), and time is a very important economic factor in the manufacture of ripened cheeses.

5.7 Pretreatment methods of Edam cheese milk: Effect on cheese yield and quality (IV)

The purpose of the cheese milk pretreatment methods, e.g. microfiltration (MF), ultrafiltration (UF), high temperature heat treatment (HH), is to increase the recovery of milk components in cheese. In this study the effects of these milk pretreatment methods for Edam -type cheeses were compared.

In MF and UF milks, lactose reduction was proportional to the CF value. The casein/whey protein (WP) ratio was increased 21% and 10% by MF and UF, respectively. However, the increased casein/WP ratio in UF milk was not detected when amounts of individual whey proteins were compared with amounts of casein. High temperature heat treatment (HH) partially denaturated whey proteins and consequently the casein/WP ratio increased by 12% due to attachment of whey proteins to casein micelles. The highest casein/α-LA and casein/β-LG ratios were observed in MF milk due to the whey protein permeability of MF membranes. Denaturation of β-LG was detected in HH milk by analyzing the casein/β-LG.

Recovery of milk total solids (TS) in cheese was highest with MF and UF, as expected. Component recovery (CR) of milk TP was highest with MF due to the high casein/total protein (TP) ratio. Elevated milk TP content did not increase CR of TP in cheese, as was also reported by Guinee et al. (2006). In addition, casein CR was at same level with all pretreatment methods. Fat component recovery (CR) was enhanced with all pretreatment methods, as was also reported by Guinee et al. (2006). Explanantion for that was probably the more intensive attachment of fat globules to protein matrix. There was a little variation in moisture contents of the fresh cheese and moisture-corrected cheese yields. The cheese yield was proportional to the casein concentration of vat milk. When yield was calculated from raw milk (ACY_r) there was a statistically significant, slightly lower yield with MF which can explained by the fact that whey proteins are removed from milk before cheese manufacture and whey proteins can not end up to cheese.

Only minor differences were detected in the composition of ripened cheese. In the MF cheese TP was elevated, but the difference was not statistically significant. In the MF cheese, casein concentration was highest and fat concentration lowest and therefore fat on a dry basis (FDB)

was lowest. The probable reason for this was the standardized milk fat/TP ratio, which actually caused an increased fat/casein -ratio after a part of milk TP (WPN) was removed with MF before the cheese manufacture. In this case the correct standardization parameter would be the fat/casein -ratio. Cheese ripening was measured by analyzing titratable fatty acids (TFA), and the results indicated that the milk pretreatment methods had significantly slowed down the ripening process, in accordance with the results of Bech (1993) and Benfeldt (2006). However, in all Edam cheeses TFA values were low and variance of the results was high. Cheese moisture of the non fat substance (MNFS) affects cheese structure and ripening (Ur-Rehman at al., 2003). In this study MNFS levels were similar and therefore it can be concluded that the pretreatment method had only a minor effect on starter activity during Edam cheese ripening. Furthermore, milk pretreatment methods had no influence on cheese acidification during the cooking step of Edam cheese manufacture.

In textural analysis of Edam cheeses, minor changes between cheeses were detected. Harder MF and UF cheeses could be obtained as a result of a more rigid coagulum, due to the higher milk protein concentration (St-Gelais et al., 1995; Neocleous et al., 2002a), and the hard HH cheese was a result of whey protein denaturation. Milk pretreatment methods did not have any influence on cheese springiness, as was reported by St-Gelais et al. (1995). However, resilience of MF and UF cheeses was significantly higher, which was probably due to the lower internal volume of the more dense protein network, and therefore this kind of cheese returns to its initial state after pressing. In sensory analyses the MF, UF and the HH cheeses were comparable and the quality of these cheeses was considered as excellent. However, differences between cheeses were negligible, indicating that with different pretreatment methods it is possible to reach the same Edam cheese specifications.

5.8 Pretreatment methods of Edam cheese milk and their effects on whey composition (V)

Use of MF and UF as milk pretreatment methods produced ca. 22% less whey compared to REF and HH processes. Amounts of MF and UF whey were identical when milk TP was 4.2%. Milk pretreatment methods had no influence on whey TS, lactose and fat contents, but TP contents were higher in MF and UF wheys compared to HH and REF wheys. Increased TP contents of MF and UF wheys were due to higher casein and WP contents, respectively. TP of MF and UF wheys were reduced by 15 and 12% compared to REF and HH wheys. Higher caseinomacropeptide (CMP) concentration was observed in MF and UF wheys, but the

caseinomacropeptide of cheese milk casein ratio was similar with all wheys which indicated that modification of milk did not have any influence to amount of CMP as observed in study II.

Milk components were divided to the MF/UF retentate and permeate after filtration, and after the cheese milk coagulation step to cheese mass and whey. MF and UF wheys contained less residual fat and 15% less total protein (TP) compared to REF or HH whey. In the case of UF whey, reduction of TP was due to NPN permeation and in the case of MF whey due to native whey protein (NWP) and NPN permeations. HH reduced WP content in whey compared to REF whey, as a result of partial heat denaturation of WP during the high temperature heat treatment. Amounts of α-LA and β-LG were significantly higher in REF whey, indicating that HH, MF and UF pretreatments were decreasing the amounts of these whey proteins in whey.

MF and UF permeates contained 28% and 24% of TS of the reference whey TS, respectively. MF permeate contained 23% and 19% of MF whey NPN and WP content, respectively. UF permeate contained 4.9% of milk TP, as described also by Maubois and Mocquot (1975). NWPC made of MF permeate contained less high molecular mass whey proteins such as BSA, lactoferrin (LF) and immunoglobulin G (IgG) compared to reference whey protein concentrate (REF WPC), but traces of casein were found in NWPC, as was also reported by Karleskind et al., (1995).

TS recoveries in MF and UF wheys were naturally reduced, since the TP of TS was increased. In addition, reduced recovery yields (RY) of fat and TP in MF and UF wheys were observed as well as reduced RY of α-LA, β-LG and WP in MF and UF wheys. The reduced RY of WP could be caused by the absence of caseinomacropeptides (CMP) in milk serum before coagulation.

The content of CMP increased in MF and UF wheys since part of TS was transferred to the permeate, but this was seen more clearly in MF whey, as expected. HH had no influence on CMP formation. However, the total amount of CMP was similar with different pretreatment methods when the total amount of casein was the same in each trial. The amount of glycosylated caseinomacropeptides (GMP) varied between 57 and 61% of caseinomacropeptides (CMP), which was in accordance with previously published data (Vreeman et al., 1986; Lieske and Konrad, 1996; Mollé and Léonil, 2005). Total amounts of caseinomacropeptides were close to the theoretical yields (Swaisgood, 2003).

As chemical analyses of MF and UF permeates showed a lack of high molecular mass whey proteins, the individual whey protein compositions of WPC powders were analyzed. Results of SDS-PAGE indicated that all WPC powders contained BSA, LF and IgG, but with this method it was not possible to quantify these proteins. In order to compare these WPC powders, the amino acid composition of each powder was analyzed and from these results it could be concluded that variation of amino acid composition was limited. The most striking difference was observed between MF WPC and REF WPC, in which the contents of the important amino acids threonine (Thr) and tryptophan (Trp) varied most. Increased content of Thr and decreased Trp content indicated a higher content of CMP in the MF WPC powder. MF with a low CF value as a milk pretreatment method has a negative effect on cheese whey quality. However, MF produces MF permeate in which the Trp content is increased and the Thr content decreased. MF is not the best milk pretreatment method if cheese whey is used as a raw material for infant formula, in which reduced levels of Thr and elevated levels of Trp are preferred (Thomä et al., 2006). However, MF permeate is an ideal raw material for infant formula and this means that if MF is used as a cheese milk pretreatment method the CF value in MF should be maximised in order to obtain the major part of whey as native whey. A high amount of native whey means the use of a high concentration factor in milk microfiltration (study I) and a low content of whey proteins in cheese milk. This intensive milk microfiltration causes changes in milk coagulation kinetics and cheese ripening in the cheese process, as described in studies III and I, respectively. Analogously, caseinomacropeptide (CMP) -enriched cheese whey also has different functional properties (Outinen and Rantamäki, 2008) and could be used especially when gelation or certain nutritional properties are required (Thomä-Worringer et al., 2006). CMP enriched cheese whey is second whey and it should be processed separately in order to realise the benefits of this microfiltration process, which causes increased complexity of whey processing.

5.9 Functional properties of whey protein concentrate powders (VI)

Reasons for lower whey protein level in NWPC powders compared to CWPC powders were lack of caseinomacropeptides (CMP) and retention of whey proteins during skimmed milk microfiltration. CMP normally represents 15-25% of cheese whey protein content (Regester and Smithers, 1991; Tolkach and Kulozik, 2004). The reason for lower fat content in NWPC powders was the double microfiltration (1.4 μm and 0.1 μm) of skimmed milk, resulting in almost total retention of fat in the casein concentrate. Only traces of fat can pass to the MF

permeate, and these traces are considered to be milk phospholipids (Phillips et al., 1990). In this study production of CWPC powders did not include the skimming step, resulting in a high amount of residual fat. High amount of residual fat affected the functional properties of CWPC powders. There was some compositional variation in NWPC powders, mainly caused by the more complex process compared to the production process of CWPC powders. NWPC-FD was manufactured in such a way that no pasteurization step was included, and the native whey protein NWP to total protein ratio varied from 81 to 85.5% of TP. It has been reported that the composition of native whey is close to that of sweet whey but without CMP, casein fines, chymosin enzyme and cheese starter residual (Maubois, 2002). In this study with ceramic microfiltration membranes this was also observed. Jost et al. (1999) concluded that large molecular mass whey proteins such as lactoferrin and immunoglobulins are retained in the retentate during milk microfiltration, and that due to this native whey and cheese whey have different protein compositions.

As expected, drying methods had only a limited effect on whey protein functional properties. Gel strength was influenced by pH, ionic strength and mineral or sugar composition of the protein mixture (Boye et al., 1995). In this study, the chemical compositions (excluding fat and CMP) of WPC powders were rather similar, and accordingly difference in chemical composition could not be the reason for the observed difference in foaming and gelation properties. The obvious reason was the lack of CMP and high content of NWP in NWPC powders (Veith and Reynolds, 2004; Outinen and Rantamäki, 2008). The reason for the more elastic gel structure obtained with NWPC powders was their lower amount of denaturated whey protein.

Presence of phospholipids and lipoproteins (Joseph and Mangino, 1988) and high amount of fat residues were the most probable reasons for lower foam stability of CWPC and foam volume of CWPC, as was also reported by Muller (1976) and Vaghela and Kilara (1996). Foam stability was much better with NWPC powders due to their high content of native whey proteins. However, NWPC powders have shown lower fat binding, poorer mouth-feel and flavour properties compared to egg white (De Wit, 1998b). In angel-cake tests WPI also resulted in poorer structural properties (Arunepanlop et al., 1996). Consequently, in this study (VI) the results obtained did not indicate that the properties of NWPC powders as food structuring agents were better than those of egg white. According to this study (VI), protein composition and solubility influenced the emulsifying capacity (EC). EC, solubility and native WP to TP ratio were the highest with NWPC powders and lowest with WPC powders.

Factors affecting EC include protein solubility, salt content, pH, other solutes (De Wit, 1988) and heat treatment history (Vaghela and Kilara, 1996). The water-holding capacities of NWPC powders and CWPC-SD were similar, but industrial WPC-SD had higher water-holding capacity due to the higher level of denaturation of whey proteins. The observed lower solubility and higher water-holding capacity of WPC-SD were in agreement with previous studies by Modler and Harwalkar (1981) and Fachin and Viotto (2005). The analysis of functional properties of native whey powders showed that native whey processing can be valuable if functional properties and purity of protein is appreciated.

6 CONCLUSIONS

On the basis of the studies presented in this thesis, the following conclusions concerning microfiltration (0.05-0.2 μm) as a cheese milk pretreatment method for cheese manufacture, and its effects on native whey and cheese whey properties can be drawn.

1. β-Lactoglobulin mass flux of skimmed milk with polymeric hollow fiber membranes was higher with higher tangential flow rates. Increase in transmembrane pressure resulted in lower permeability of whey proteins. Polymeric spiral wound microfiltration membranes had lower energy consumption, higher retention of β-lactoglobulin and α-lactalbumin to milk retentate and lower permeate flux values compared to ceramic membranes. Polymeric microfiltration membranes were acceptable alternative for ceramic membranes on skimmed milk microfiltration.

2. Optimized milk for Emmental cheese includes at least 3.2% lactose in milk to guarantee cheese pH changes and ripening. Microfiltration with high concentration factor values (CF 10.8) increased ripening rate of Emmental cheese due to lower contents of plasmin inhibitors such as native whey protein and ash. Microfiltration of milk does not increased cheese yield.

3. Reduction of milk whey protein, lactose and ash content did not influence chymosin activity. Reduction of whey protein content in cheese milk increased the caseinomacropeptide (CMP) portion in the total proteins in cheese whey. The CMP content of whey total protein content increased up to 40% when CF 10.8 was used.

4. Milk coagulation properties were largely influenced by milk pH, $CaCl_2$ and chymosin additions, and by the content of milk protein. Rennet clotting time was decreased when lactose, whey protein and ash content in milk were reduced. In addition hardness of the coagulum was increased and the time difference between rennet clotting and optimum cutting time was shortened. Changes in milk coagulation kinetics were based on faster diffusion of rennet (chymosin), lower filling material content in milk and lower milk ash content. Decreased whey protein and salt contents in milk also increased chymosin activity. Due to these reason it can be concluded that microfiltration of cheese milk changed milk coagulation kinetics.

5. Cheese yield calculated from raw milk to cheese was lower with microfiltrated milks, due to reduced amounts of whey proteins: casein recovery from vat milk to cheese did not vary significantly. However, elevated recoveries of fat, total protein and total solids from vat milk to cheese were observed. MF and UF cheeses with similar moisture content were harder than reference cheeses. Cheese hardness increased when microfiltration and ultrafiltration resulted in elevated milk protein concentration. Due to the changed cheese protein network, higher cheese resilience was obtained from cheeses made of microfiltrated and ultrafiltrated milk. However, cheeses from microfiltrated and ultrafiltrated milk were considered as more pleasant compared to the reference cheese. It can be concluded that microfiltration or ultrafiltration as a pretreatment method for Edam cheese milk has no negative influence on cheese manufacture.

6. Microfiltration-produced native whey (MF permeate) contained part of the milk whey proteins, and the contents of native whey depended on the concentration factors (CF) used. Microfiltration permeate did not contain as much higher molecular mass whey proteins as cheese whey. A low amount of whey proteins in cheese whey resulted in a new type of CMP-enriched whey. The amino acid composition of native WPC differed from that of WPC made from cheese whey, mainly due to the lower threonine (Thr) and higher tryptophan (Trp) contents. In fact, even at low CF values (CF 1.4), skimmed milk microfiltration affected the amino acid composition of cheese whey and changed its nutritional value. Ultrafiltration as a milk pretreatment method did not change cheese whey composition, because during milk coagulation the whey protein to casein ratio did not change. Total masses of α-LA and β-LG were significantly higher in the reference (REF) whey, indicating that HH, MF and UF milk pretreatments decreased the total mass of these whey proteins in whey.

7. Powders from native whey protein concentrate had excellent functional properties as well as different amino acid compositions. Whey protein concentrate powder functionality was not dependent on the drying method, but on the process history and the source and content of protein concentrate.

Microfiltration is beneficial for the cheese manufacturer because it allows optimization of the composition of cheese milk. This means a lower amount of additives (chymosin, starters), higher recovery yield of fat and protein from vat milk to cheese, standardized protein content of vat milk and lower need for additional water during cooking for lactose removal. For whey processing the microfiltration also gave some benefits. A lower amount of water addition during the cheese cooking phase reduced the amount of cheese whey and increased whey total solids content, which therefore decreased the need to concentrate the whey. Native whey can be utilized as a raw material for infant formula because it has a higher tryptophan and lower threonine content than cheese whey due to its lack of caseinomacropeptides. Whey protein products made from native whey had better functional properties such as gelation and foaming properties, and they allow utilization of native whey proteins as food structuring agents. In addition caseinomacropeptide-enriched cheese whey can be a raw material for products in which aromatic amino acids are not desired. Microfiltration is also a very promising alternative because native whey can be obtained from milk before the cheese process without lactic acid formation in whey. Thus microfiltration creates possibilities to utilize milk whey components in the best possible way in other processes. Microfiltration can be used for cheese milk modification and it is possible to standardize ideal cheese milk for each cheese type. Ideal cheese milk contains important milk components for cheese manufacture, and milk components which are unnecessary for the cheese process can be removed with the native whey.

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8 APPENDIX A (ORIGINAL PAPERS I-VI)