International Dairy Journal 97 (2019) 222–229

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Pilot-scale β-casein depletion from micellar casein via cold microfiltration in the diafiltration mode

Johannes Schäfer [1], Thomas Schubert [1], Zeynep Atamer*

University of Hohenheim, Institute of Food Science and Biotechnology, Department of Soft Matter Science and Dairy Technology, Garbenstr. 21, D-70599, Stuttgart, Germany

ARTICLE INFO

Article history:
Received 9 February 2019
Received in revised form
12 May 2019
Accepted 18 June 2019
Available online 12 July 2019

ABSTRACT

The aim of this study was to produce pilot scale batches of β-casein concentrate and micellar casein concentrate with reduced β-casein level. The isolation of β-casein was done using membrane filtration at cold temperatures (≤5 °C). A micellar casein concentrate was obtained from skim milk by means of warm microfiltration (MF) at 50 °C (0.1 μm pore size, ceramic membranes). The concentrate was stored at 2 −3 °C for approximately 40 h to induce temperature-dependent dissociation of β-casein from casein micelles. β-casein was separated from the cold-stored concentrate using MF (0.3 μm pore size, organic membranes) at ≤5 °C. β-Casein permeate was warmed up to 50 °C to lead self-association of β-casein micelles before ultrafiltration at 50 °C (10 kDa cut-off, organic membranes). Two streams, a β-casein-depleted and a β-casein concentrate, were generated. A purity of 92.64% and a yield of up to 18.07% were achieved for β-casein.

1. Introduction

During the production of fermented protein-enriched dairy products such as fresh cheese and “Greek style” yoghurt, a centrifugation or a membrane filtration [microfiltration (MF) or ultrafiltration (UF)] process is usually applied to increase the dry matter. The concentration process can be done either before or after fermenting the milk (Schäfer et al., 2019). In the latter, fermentation-concentration process (FCo), acid whey is an undesired by-product, whose valorisation is difficult (Konrad, Kleinschmidt, & Faber, 2012; Shon & Haque, 2007). By means of a concentration-fermentation process (CoF), during the concentration step via microfiltration of skim milk, the mineral and the protein contents increase. The MF-permeate obtained, which is free of cheese fines, fat, salt, rennet, starter culture, colour and glycomacropeptide, represents a virgin whey protein stream (Ardisson-Korat & Rizvi, 2004) and is often referred to as “ideal whey” (Lawrence, Kentish, O'Connor, Barber, & Stevens, 2008). An elevated mineral content, especially calcium (Flüeler & Puhan, 1977; Friis, 1981; Patel, Reuter, & Prokopek, 1986), and the presence of bitter peptides at high concentrations formed by hydrolysis of milk proteins via proteinases (e.g., cathepsin D, lactococcal proteinases) can cause sensory defects in the final product (Sebald, Dunkel, Schäfer, Hinrichs, & Hofmann, 2018).

Our preliminary work has already shown that calcium reduced CoF fresh cheese samples showed a reduction in the bitterness, however, the bitterness increased slightly during storage, indicating further hydrolysis of (β-)caseins during storage. Sebald et al. (2018) identified 17 bitter peptides in CoF fresh cheeses and reported that the peptides from β-casein revealed a primary contribution to the perceived bitter taste of fresh cheese samples. In the light of our recent observations (Schäfer et al., 2019) and the literature showing the presence of bitter peptides arising from the hydrolysis of β-casein (Baum, Fedorova, Ebner, Hoffmann, & Pischetsrieder, 2013; Sebald et al., 2018), reduction of bitter peptide content as well as their further release during storage can be taken as a strategy to prevent or reduce the sensory bitterness of the fermented dairy products made of milk retentates.

Several attempts have been made for the separation of the casein fractions (α-, β-and κ-casein), the applied methods were summarised in detail by Atamer et al. (2017), which includes basically selective precipitation as well as membrane filtration at low temperatures. In order to alter the amount of β-casein in micellar casein concentrates, MF membranes have been used

* Corresponding author. Tel.: +49 711 45923646.
E-mail address: zeynep.atamer@uni-hohenheim.de (Z. Atamer).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.idairyj.2019.06.009
0958-6946/

(Seibel, Molitor, & Lucey, 2015). Unlike the other two main fractions of casein, α- and κ-casein, the dissociation of β-casein from the micelle is temperature dependent (Creamer, Berry, & Mills, 1977; Downey & Murphy, 1970; Huppertz et al., 2006). β-casein dissociates from the casein micelle into the serum phase upon cooling of milk to the temperatures below 5 °C, which is a reversible process. Using MF with polymeric membranes, β-casein can be separated from skim milk at cold temperatures (Crowley et al., 2015; McCarthy, Wijayanti, Crowley, O'Mahony, & Fenelon, 2017; O'Mahony, Smith, & Lucey, 2007; Seibel et al., 2015). In this study, the aim was to study a membrane process in detail that would yield a concentrate enriched in β-casein and a micellar casein concentrate with reduced amount of β-casein on a technical scale. The second stream should serve as raw material for manufacturing CoF fresh cheese with reduced bitterness in the future.

2. Materials and methods

2.1. Production of micellar casein concentrates from raw bovine milk

Fig. 1 illustrates in detail the production steps for the manufacture of casein concentrates, β-casein and β-casein-depleted. Raw bovine milk was supplied from the research station Meiereihof (Universität Hohenheim, Stuttgart, Germany). After pasteurisation (74 °C, 30 s) and separation of fat at 55 °C (SA 10; Frautech S.r.l., Schio, Italy), the pasteurised skim milk was standardised to a protein content of 3.4%, as described by Körzendörfer, Nöbel, and Hinrichs (2017), using reconstituted ultrafiltered skim milk permeate, which consisted of 5.2% (w/w) permeate powder (Bayolan PT; BMI e. G., Landshut, Germany) and demineralised water. Skim milk was concentrated using an MF process at 50 °C (Kersten, 2001; Fig. 1A) by means of a cross-flow membrane filtration unit (Model TFF; Pall GmbH, Dreieich, Germany) equipped with multichannel gradient of permeability (GP) ceramic membranes (7P19-40 GP Membralox® Module, 99.7% α-alumina; Pall Exekia, Bazet, France) with 4-mm-diameter channels, a cut-off of 0.1 μm and an effective filtration area of 1.69 m^2.

To reduce the whey protein content, the skim milk retentate was subjected to an MF in the diafiltration (DF) mode (MF-DF) process (11 repetitive DF stages) using demineralised water (20 kg) as described by Thienel et al. (2018). The whey protein reduced retentate (micellar casein concentrate) obtained was diluted (dilution ratio = 1:4) to increase dissociation of β-casein from the micelle, which resulted in a final protein concentration of approximately 1.5% protein. After cooling, the diluted concentrate was kept at 2–3 °C for approximately 40 h to induce the dissociation of β-casein from the casein micelle. The cold stored micellar casein concentrate was subjected to a cold MF-DF process at ≤ 5 °C (MMS AG Membrane Systems, Urdorf, Schweiz, equipped with two spiral-wound membrane modules (Dairy JX series 3840C50, polyvinylidene fluoride; General Electric, Boston, MA, USA) with a filtration area of 2 × 5.1 m^2 and a pore size of 0.3 μm), which resulted in two streams: β-casein-depleted retentate 3 and β-casein enriched permeate 3 (Fig. 1A). The obtained retentate was then diluted (dilution ratio = 1:2) and stored at temperatures below 5 °C for 2 h in situ. The diluted retentate was again subjected to a cold concentration process by means of MF (pore size: 0.3 μm) at ≤ 5 °C, which delivered β-casein-depleted retentates 4 and 5 as well as β-casein enriched permeates 4 and 5.

The β-casein permeates (3, 4 and 5) obtained were collected in a tank (Fig. 1B) and heated to 50 °C and kept at this temperature for approximately 1 h to induce self-association of β-casein. The combined permeates were subjected to warm ultrafiltration process at 50 °C (MMS AG Membrane Systems, Urdorf, Schweiz, equipped with two spiral-wound membrane modules (KMS 3838 HFK-131, polyethersulfone; Koch Membrane systems, Wilmington, MA, USA) with a filtration area of 2 × 6.7 m^2 and a pore size of 10 kDa) to further enrich the β-casein. Subsequently, the obtained retentates (Ret 5 and Ret 6) were spray- and freeze-dried. The experiment was done in triplicate.

2.2. Analysis

2.2.1. Major constituents of casein concentrates

The measurements of total protein, calcium, dry matter, lactose and fat contents were carried out according to the corresponding methods listed in our previous study (Schubert, Meric, Boom, Hinrichs, & Atamer, 2018). The protein content was determined by a nitrogen analyser (Dumatherm-DT, C. Gerhardt GmbH & Co. KG, Königswinter, Germany) following the ISO 1489|IDF 185:2002 Dumas method (ISO, 2002). A conversion factor of 6.38 for milk and milk proteins was used. The fat content was determined according to the method of Gerber VDLUFA C 15.3.6 (VDLUFA, 2011). Calcium content was determined by complexometric titration with ethylenediaminetetraacetic acid (EDTA) according to VDLUFA C 10.6.8 (VDLUFA, 2011). Lactose content of the micellar casein concentrates was determined by an accredited lab (Landwirtschaftliches Zentrum Baden-Württemberg (LAZBW), Wangen, Germany) according to an enzymatic method (DIN EN ISO/EC 17025:2005; DIN, 2005). The dry matter content was determined according to the method of the sea sand method (C 35.3; VDLUFA, 2011). Water content of the powders (β-casein and β-casein-depleted powders) were determined by Karl-Fischer titration according to the method proposed in DIN EN ISO 12779:2013-08 (DIN, 2013), using an automatic titrator (Titrando 841, Deutsche Metrohm GmbH&Co KG, Filderstadt, Germany). All analyses were performed in triplicate.

2.2.2. Reversed-phase high-performance liquid chromatography

The total casein content and casein composition were determined as described in detail by Schubert et al. (2018) using reversed-phase high performance liquid chromatography (RP-HPLC) (Agilent 1260 Infinity, Agilent Technologies System, Santa Clara, CA, USA), equipped with a quaternary pump (Agilent 1260 Series, G1311B), auto sampler (Agilent 1260 Series, G1329B), thermostat with a column compartment (Agilent 1290 Series, G1330B; Agilent 1260 Series, G1316B) and a variable wavelength UV/VIS detector (Agilent 1260 Series, G1315D). Casein standards, C6780 (α-casein), C6905 (β-casein) and C0406 (κ-casein), were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as standards. Whey protein content was determined via RP-HPLC according to ISO 13875 IDF 178:2005 (ISO, 2005). All analyses were performed in triplicate.

2.3. Particle size measurement

The particle size measurement of the feed, retentate and permeate samples (see also Table 1 and Fig. 1) taken during the manufacturing process was carried out by means of dynamic light scattering (Zetasizer Nano Series DS; Malvern Instruments, Worcestershire, UK) according to Schäfer et al. (2018). Samples were tempered to the respective processing temperature (ϑ = 50 °C and approximately 5 °C) using a water bath (CC1, Peter Huber Kältemaschinenbau AG, Offenburg, Germany) or iced water. Permeate and Feed 3 samples (aliquot volume = 2000 μL) could directly be measured, whereas Feed 2 and retentate 5 and 6 samples (aliquot volume = 20 μL) were diluted in equally tempered demineralised water (dilution medium volume = 2000 μL). The particle size was expressed as the intensity weighted mean hydrodynamic diameter z-average. For the calculation of the volume

based particle size distribution according to the Mie theory refractive indices of water and casein of 1.33 and 1.57 were used, respectively. Furthermore, viscosities of water at 5 °C and 50 °C of 1.4774 and 0.5482 mPa s were used, respectively. Duplicate measurements were performed for each sample.

2.4. Scanning electron microscope

The structures of the manufactured powders of micellar casein concentrates were visualized with a scanning electron microscope (Jeol, JSM-IT100 InTouchScope™, USA) for studying surface appearance of produced powders.

2.5. Calculations

The purity of a casein fraction ($P_{n\text{-}CN}$, %) or the casein composition of a sample ($C'_{n\text{-}CN}$, %) was defined as the ratio of the casein fraction content ($c_{n\text{-}CN,\ fraction}$) or the amount of a casein fraction or fractions ($\sum c_{n\text{-}CN,\ fraction}$) and the total casein content in the isolated fraction/sample ($c_{total\ CN,\ fraction}$) (Eq. (1)) respectively.

$$P_{n-CN}\ or\ C'_{n-CN} = \left(\frac{\sum c_{n-CN,\ fraction}}{c_{total-CN,\ fraction}}\right) \cdot 100\ \% \quad (1)$$

The relative enrichment or reduction of one or more fractions ($E_{n\text{-}CN}$, %) was defined as the ratio of the purity of the casein fraction and the purity of the fraction in the raw material ($P_{n\text{-}CN,\ Feed\ 1}$) minus 1 (Eq. (2)).

$$E_{n-CN} = \left(\frac{P_{n-CN,\ fraction}}{P_{n-CN,\ Feed\ 1}} - 1\right) \cdot 100\ \% \quad (2)$$

The yield ($Y_{\beta\text{-}CN,\ }$%) for the β-casein fraction is defined as the ratio of the amount of the obtained β-casein fraction to the total amount of β-casein fraction in the raw material (Feed 1) and was calculated as follows:

$$Y_{\beta-CN/total\ \beta-CN} = \frac{m_{\beta-CN,\ fraction} \cdot P_{\beta-CN}}{m_{Feed\ 1} \cdot c_{\beta-CN,\ Feed\ 1}} \cdot 100\ \% \quad (3)$$

where $m_{\beta\text{-}CN,\ fraction}$ refers to the mass of the obtained β-casein fraction, $m_{Feed\ 1}$ to the initial mass of raw material (Feed 1) used for the isolation and $c_{\beta\text{-}CN,\ Feed\ 1}$ refers to the total β-casein content (as determined by RP-HPLC). $m_{\beta\text{-}CN,\ fraction}$ was calculated by multiplying the obtained mass of Ret 6 with the measured casein content of the obtained retentate.

The mass reduction ratio (MRR) of the filtration experiments is the ratio of the (initial) feed mass to the retentate mass at the end of the filtration was calculated as follows:

$$MRR = \frac{m_{feed}}{m_{feed} - m_{permeate}} \quad (4)$$

where m_{feed} refers to the feed mass and $m_{permeate}$ to permeate mass in kg.

3. Results and discussion

3.1. Production of micellar casein concentrates

The application of MF membranes under cold conditions (<5 °C) was proposed in different studies for the isolation of β-casein from caseinate solution or skim milk (Famelart & Surel, 1994; Hoffmann, Johannsen, & Stroebel, 2006; van Hekken & Holsinger, 2000). High purities above ~90% were achieved (i.e., Famelart and Surel (1994)). However, the yields obtained did not exceed 20% (see also Table 3). The separation of β-casein by means of cold filtration is influenced by several factors such as temperature during the filtration process, time for recirculation of the MF medium (Maubois & Ollivier, 1992) and the conditions before filtration (e.g., properties of the feed such as heat treatment parameters of milk and, viscosity) (Zulewska, Kowalik, & Dec, 2018) and need to be investigated further. Furthermore, pH of the medium, ionic strength as well as protein concentration are also the additional parameters effecting the dissociation of the β-casein from the micelle (Pierre & Brule, 1981; Pouliot et al., 1994). One of the aims of this study was to increase the obtained yield in cold filtration while having high purities, therefore the parameters, filtration temperature and time as well as dissociation time, were considered in the present study while designing the filtration process.

The productions steps for the isolation of β-casein and the casein concentrates from bovine milk are illustrated in detail in Fig. 1. During manufacture of the micellar casein concentrates, samples were collected from critical checkpoints of the production. These points are marked in Fig. 1 (points from 1 to 17). The samples were analysed for their casein, whey protein, total protein, calcium, dry matter, ash, fat and lactose contents. The chemical compositions of selected samples are summarised in Table 1. The chromatographic profiles of the obtained casein powders (β-low-MCN and β-casein), skim milk (Feed 1) and a micellar casein concentrate (Ret 2) are shown in Fig. 2. The achieved casein contents from skim milk (total casein content: 2.69 ± 0.11%) in the sampling points 13 and 17 were 82.82 ± 2.14% and 93.99 ± 5.36%, respectively. The calculated purity and yield values for β-casein were 92.64 ± 0.53% and 14.52 ± 3.85%, respectively (see Table 2). The yield for the 1st, 2nd and 3rd production varied and were 10.42%, 15.06% and 18.07%, respectively. During the first production the storage tank was heated quickly and the inner wall temperature reached temperatures between 65 and 70 °C. An ultrathin layer of β-casein (not visible with the naked eye) formed and subsequently was not available during the concentration step and caused the reduced yield during the first trial. Thus, during the 2nd and 3rd production trials, the inner wall temperature was limited to ~50 °C and consequently, higher yields were obtained.

The data from the present study was included in Table 3 with several examples of data from the literature, in which both methods, selective precipitation and membrane filtration at low temperatures, were used for the isolation of β-casein from caseinates or micellar casein concentrates. The achieved purity of 90% is in comparable magnitude with other reported purities in the previous studies (e.g., Famelart & Surel, 1994; Hoffmann et al., 2006). Although purification of β-casein up to 96% was realised, difficulties of maintaining a low temperature in the system were reported. A decrease in flux was shown with increasing viscosity of the retentate, leading to the conclusion that concentration polarisation and fouling result in low recoveries, especially at high process volumes (Hoffmann et al., 2006; van Hekken & Holsinger, 2000). With the proposed process, a yield of 18.07% was achieved without optimisation. Though there are several possibilities to further increase the yield i.e. by increasing the number of DF steps during the cold MF.

In the present study, the micellar casein concentrate obtained (protein content: 5.78%) was diluted with water (dilution: 1:4, final protein concentration = 1.5%), which increased the dissociation of β-casein from the micelle (McCarthy et al., 2017). At this protein concentration, the concentrate was kept at ≤ 5 °C for approximately 40 h, which has also an important effect on the dissociation of β-casein into the serum phase. After the isolation of β-casein, the streams containing β-casein (β-CN permeate 3, 4 and 5, Fig. 1B) were blended and with an increase of the temperature to 50 °C, the self-association of β-casein occurred and the micelles were formed

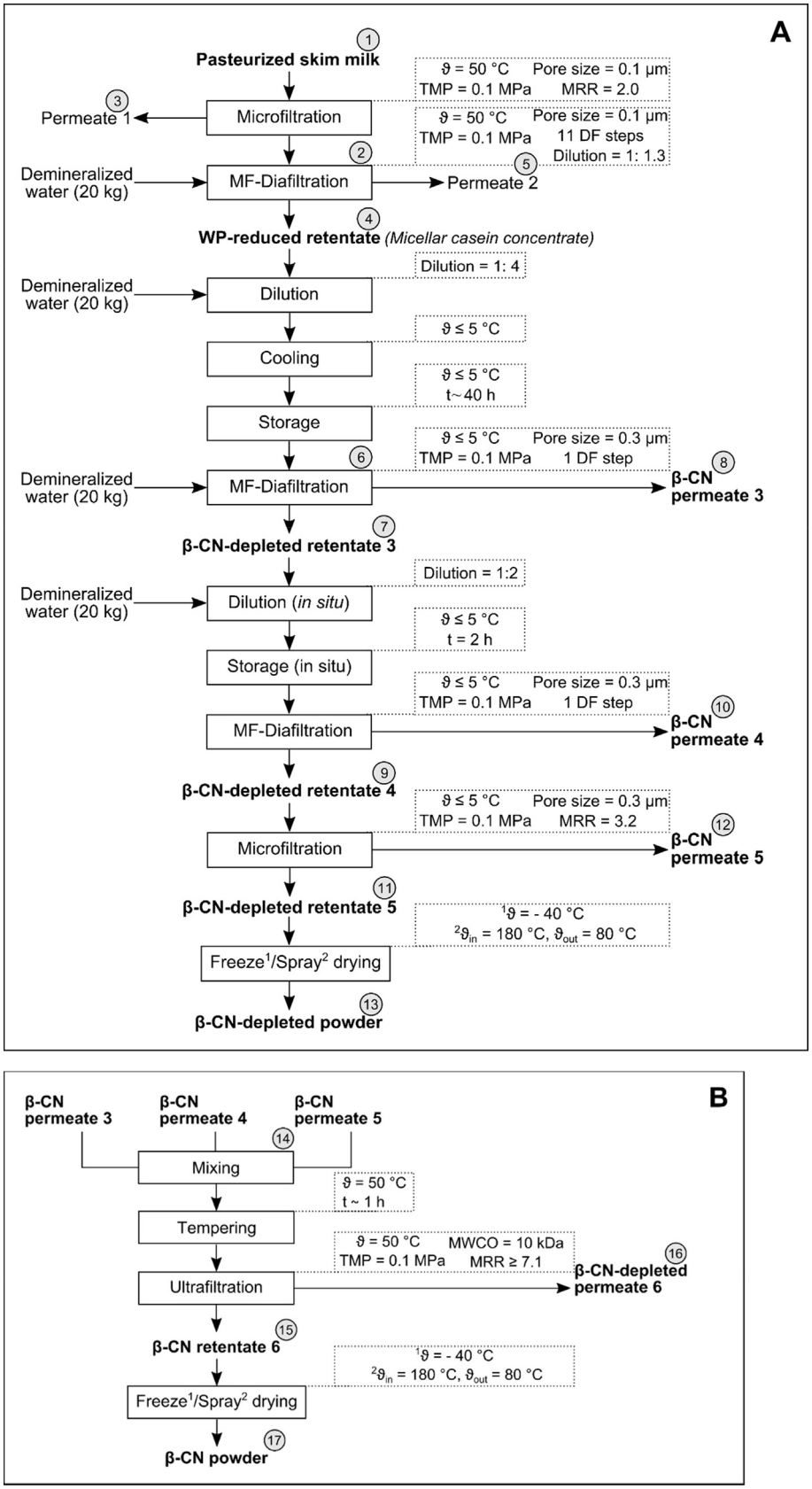

Fig. 1. Production of micellar casein concentrates from bovine milk showing the sampling points (circled numbers): (A) β-casein-depleted powder (β-low-MCN) and (B) β-casein-enriched powder (β-CN). Abbreviations are: c_p, protein content; ϑ, temperature; TMP, transmembrane pressure difference; MRR, mass reduction ratio (the ratio of the initial feed mass to the retentate mass at the end of the filtration, Eq. (4)); t, time; MF, microfiltration; WP, whey protein; CN, casein; MWCO, molecular mass cut-off.

Table 1
Chemical composition of the selected samples during manufacturing of micellar casein concentrates.[a]

Sampling point	Sample name	Composition (%, w/w)						
		Total protein	Casein	Dry matter	Fat	Lactose	Ash	Ca^{++}
1	Feed 1	3.87 ± 0.04	2.69 ± 0.11	9.26 ± 0.05	0.05 ± 0.01	n.d.	0.73 ± 0.01	0.125 ± 0.002
4	Ret 2	5.78 ± 0.11	5.30 ± 0.24	6.38 ± 0.06	<0.05	n.d.	0.14 ± 0.01	0.179 ± 0.001
6	Feed 2	1.98 ± 0.18	n.d.	1.49 ± 0.03	n.d.	n.d.	n.d.	0.046 ± 0.001
11	Ret 5	7.28 ± 0.57	6.69 ± 1.03	7.87 ± 0.63	0.11 ± 0.03	n.d.	0.68 ± 0.08	0.228 ± 0.021
13	β-low-MCN	82.82 ± 1.19	81.07 ± 4.00	91.82 ± 2.01	n.d.	0.63 ± 0.04	n.d.	2.769 ± 0.021
14	Feed 3	0.61 ± 0.08	n.d.	n.a.	<0.05	n.d.	n.d.	0.004 ± 0.001
15	Ret 6	1.60 ± 0.51	0.87 ± 0.26	n.a.	<0.05	n.d.	0.04 ± 0.01	0.014 ± 0.004
17	β-CN	83.31 ± 2.14	93.99 ± 5.36	91.42 ± 1.38	n.d.	5.51 ± 0.67	n.d.	1.378 ± 0.098

[a] Sampling points are shown in Fig. 1. Abbreviations are: Ret, retentate; MCN, micellar casein; β-low-MCN; β-casein-depleted micellar casein; CN, casein; n.a., not applicable; n.d., not determined. Values represent the means and the standard deviations obtained from three independent productions (i = 3, n = 3). Dry matter for β-low-MCN and β-CN calculated by subtracting the water content as determined by Karl Fischer titration from 100%.

Table 2
The casein contents of the obtained samples and the relative enrichment or reduction of the fractions (β-casein or α_S+κ-casein).[a]

Sampling point	Sample name	Casein content (%, w/w)				Casein composition (%)		Relative enrichment or depletion (%)	
		α_S-CN	β-CN	κ-CN	Total-CN	α_S+κ-CN	β-CN	α_S+κ-CN	β-CN
1	Feed 1	1.06 ± 0.08	1.26 ± 0.08	0.37 ± 0.02	2.69	53.16	46.84	n.a.	n.a.
4	Ret 2	2.15 ± 0.04	2.28 ± 0.15	0.88 ± 0.06	5.30	57.07	42.93	n.a.	n.a.
11	Ret 5	2.98 ± 0.34	2.51 ± 0.53	1.20 ± 0.14	6.69	62.48	37.52	17.53	−19.89
13	β-low-MCN	36.06 ± 1.85	32.10 ± 1.23	12.91 ± 2.19	80.07	60.40	39.60	16.63	−15.47
15	Ret 6	0.04 ± 0.01	0.81 ± 0.25	0.03 ± 0.01	0.87	7.28	92.72	−86.31	97.95
17	β-CN	3.89 ± 0.41	87.01 ± 5.42	3.10 ± 0.30	93.99	7.43	92.57	−86.02	97.62

[a] Abbreviations are: Ret, retentate; MCN, micellar casein; β-low-MCN, β-casein-depleted micellar casein; CN, casein; n.a, not applicable. Values represent the means and the standard deviations obtained from three independent productions (i = 3, n = 3). Casein composition was calculated according to Eq. (1). Relative enrichment or depletion was calculated according to Eq. (2), where positive values signify an enrichment of the casein fraction, whereas negative values signify a depletion of the casein fraction, when compared with the raw material (Feed 1).

Table 3
Some examples of processes for isolates of food-grade β-casein from caseinates and micellar casein.[a]

Casein raw material	Process volume	Process parameters	β-casein purity (%)	β-casein yield (%)	Reference
Membrane filtration					
0.25–3% sodium caseinate solution	1 L	UF at 4 °C, 100 kDa, 300 kDa, 0.1 μm	70–80	3–20	Murphy & Fox (1991)
1% sodium caseinate solution	45 L	UF at 4 °C, 20.5 nm, 28.2 nm, 80.0 nm	≤60	4	Le Berre & Daufin (1994)
2% sodium caseinate solution	0.2 m^2 MF plant	MF at 4 °C, 0.1 μm, 0.2 μm, 0.5 μm	39–96	2–10	Famelart & Surel (1994)
2% sodium caseinate solution	80 kg	MF at < 3 °C, 0.1 μm	86	14(1)	Hoffmann et al. (2006)
2.7% micellar casein solution	140 kg	MF at 5 °C, 0.3 μm and UF at 50 °C, 10 kDa	>90(1)	10–18(3)	Present study
Centrifugation using selective solubility and precipitation					
Renneted skim milk	0.5–8 kg	Clarifier centrifuge, decanter, 2–5 °C	> 90	–	Le Magnen & Maugas (1995)
Renneted skim milk	200 mL	Centrifuge, 5 °C	–	~20(1)	Huppertz et al. (2006)
2.7% sodium caseinate solution	200 mL	Centrifuge	>95	–	Law & Leaver (2007)
2.7% micellar casein solution	200 mL	Centrifuge, 4 °C	95	15	Post, Ebert, & Hinrichs (2009)
2.7% sodium, potassium, and calcium caseinate solutions	200 mL	Centrifuge, 4 °C	91–96	2–3	Post & Hinrichs (2010)
3.1% micellar casein solution	85 L	Centrifuge, 4 °C	85	8	Post & Hinrichs (2011)
7% micellar casein solution	20 kg	Decanter, 4 °C	95(2)	19(2)	Schubert, Meric, Boom, Hinrichs, & Atamer (2018)

[a] Modified from Atamer et al. (2017). Abbreviations are: MF, microfiltration; UF, ultrafiltration, DF, diafiltration. Superscript parenthesised numbers indicate: (1) values given for recovery of β-casein; (2) measured values; (3) calculated value according to Eq. (3).

(hydrodynamic diameter z-average of the micelle at 50 °C = 183.9 ± 45.1 nm, see section 3.2). The feed containing formed micelles could be enriched via UF (cut off = 10 kDa) at 50 °C (protein content of the retentate = 1.60%, Table 1).

Cold filtration was proposed not only for the enrichment of β-casein but also for the alteration of relative β-casein content to the other casein fractions, α- and κ-casein, in cheese making (O'Mahony, McSweeney, & Lucey, 2008; O'Mahony et al., 2007). O'Mahony et al. (2007) patented a three-stage membrane filtration process. They produced a demineralised, lactose-free, soluble β-casein from skim milk with a purity of >90% as well as a β-casein depleted milk. The β-casein depleted milk was tested for the production of cheese and it was observed that the cheese had increased melting abilities (O'Mahony et al., 2008).

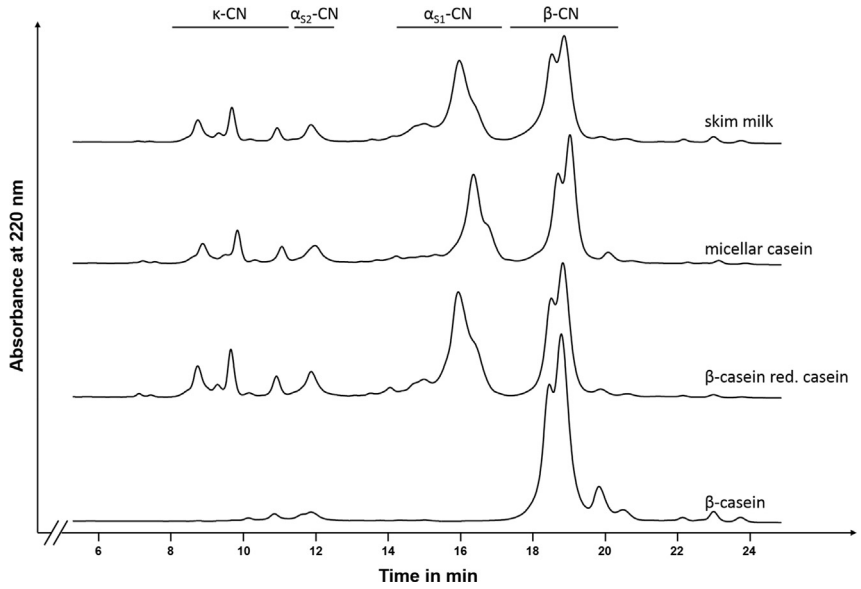

Fig. 2. Reversed-phase high performance liquid chromatography profiles of skim milk (Feed 1, sampling point 1), micellar casein concentrate (Ret 2, sampling point 4), β-casein-reduced casein powder (β-low-MCN, sampling point 13) and β-casein powder (β-CN, sampling point 17).

3.2. Particle size measurements of the micellar casein concentrates and visual characterisation of the powders

The particle size distributions of the samples, micellar casein concentrate (sampling point 6, see also Fig. 1), β-casein-depleted concentrate (sampling point 11) and β-casein concentrate (sampling point 15), were measured. The measurements were carried out under cold ($\vartheta = 5$ °C) and warm filtration conditions ($\vartheta = 50$ °C). The results are given in Fig. 3. The particle size distributions of the micellar casein and β-casein-depleted concentrates were similar. As expected, much smaller particle sizes were observed in the β-casein concentrate. As mentioned in section 3.1, the filtration temperature was increased to 50 °C to induce the formation β-casein micelles and therefore to enable the enrichment of β-casein via UF (Fig. 1B, sampling point 14, see also Fig. 4). With increasing temperature (from 5 to 50 °C), micelles were formed from monomers (hydrodynamic diameter z-average = 48.4 ± 5.7 nm, $\vartheta = 5$ °C) and stabilised by hydrophobic interactions (Lucey, Srinivasan, Singh, & Munro, 2000). The measured sizes of the formed aggregates (permeate containing β-casein) are shown in Fig. 4 (hydrodynamic diameter z-average = 183.9 ± 45.1 nm, $\vartheta_2 = 50$ °C, image B, first warming). The micelle aggregation was also observed with naked eye (compare the images A (clear) and B (turbid) in Fig. 4), which was also described in the literature (Bachar et al., 2012; Moitzi, Portnaya, Glatter, Ramon, & Danino, 2008). This process was partly reversible and upon cooling to 5 °C, dissociation of β-casein was observed, as it was also previously reported (Creamer et al., 1977; Downey & Murphy, 1970). The measured sizes of the particles are given in Fig. 4 (hydrodynamic diameter z-average = 62.3 ± 24.7 nm, $\vartheta_3 = 5$ °C, image C). As the same

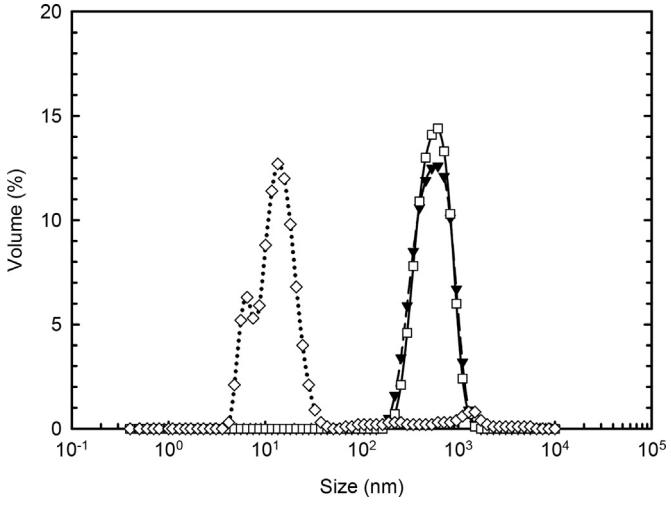

Fig. 3. Particle size distribution of the samples from the following sampling points measured at $\vartheta = 5$ °C: ▼, micellar casein concentrate (Feed 2, sampling point 6); □, β-casein-depleted concentrate (Ret 5, sampling point 11); ◇, β-casein concentrate (Ret 6, sampling point 15). Mean values from two independent experiments are shown (i = 2; n = 2).

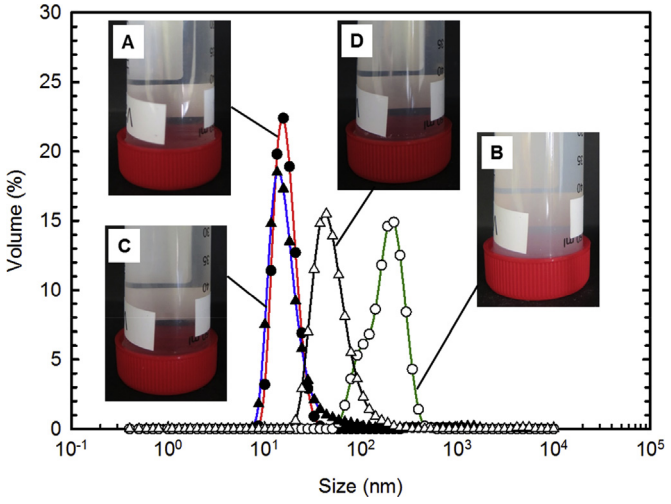

Fig. 4. Particle size distribution and images (A–D) of permeate containing β-casein (Feed 3, sampling point 14 (Fig. 1)) subjected to cooling–warming cycles: ●, $\vartheta_1 = 5$ °C (A); ○, $\vartheta_2 = 50$ °C (B); ▲, $\vartheta_3 = 5$ °C (C); △, $\vartheta_4 = 50$ °C (D). Mean values from two independent experiments are shown (i = 2; n = 2).

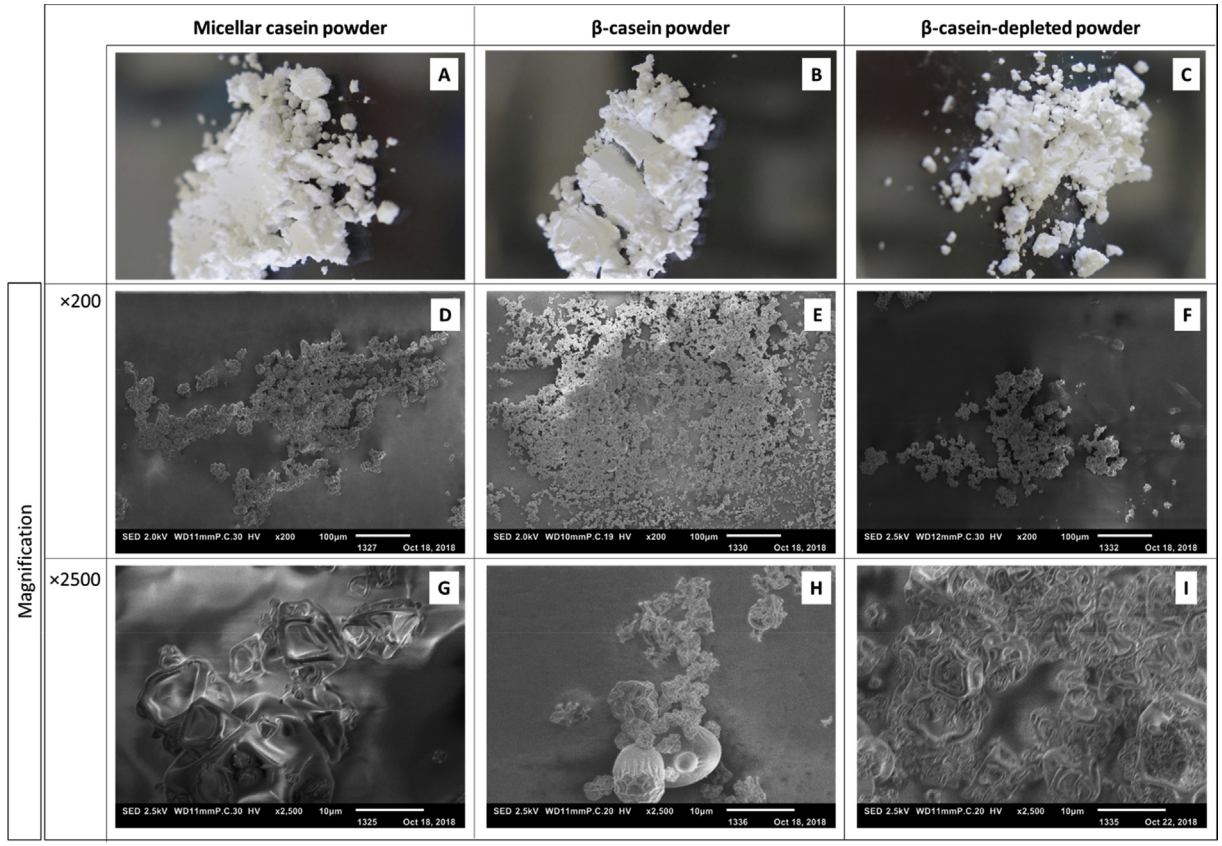

Fig. 5. Images of manufactured: A, spray-dried micellar casein concentrate powder (Ret 2, sampling point 4); B, β-casein powder (β-CN, sampling point 17); C, β-casein-depleted powder (β-low-MCN, sampling point 13) together with the scanning electron microscopic images of micellar casein powder (D, G) β-casein powder (E, H) and β-casein-depleted powder (F, I).

permeate containing β-casein was warmed up to 50 °C again, a formation of micelle was observed again, although the sizes of the formed micelles were smaller in the second warming process. The measured particle size and the observed image are also illustrated in Fig. 4 (hydrodynamic diameter z-average = 68.0 ± 23.7 nm, $\vartheta_4 = 50$ °C, image D, second warming), which was not turbid due to the smaller formed micelle sizes (compare the ones formed in the first warming, image B).

The obtained concentrates were dried using a spray drier to see the effect of drying process on the produced concentrates and to get first impression on the produced powders regarding their microscopic and macroscopic properties. Therefore, besides the particle size measurements, the surface appearance of the produced powders was analysed. Fig. 5 shows the images of the spray-dried micellar casein, β-casein and β-casein-depleted powders as well as their SEM images with two different magnifications (200× and 2500×).

4. Conclusion

Micellar casein concentrate with reduced amount of β-casein (15.5% reduced β-casein content) and pure β-casein concentrate ($P_{\beta\text{-CN}} = 92.64\%$) were manufactured using a combination of warm MF, cold MF-DF and warm UF from bovine milk and the production process was described in detail. Highly purified β-casein could be obtained using the process, the obtained yield was comparable to previous findings (Schubert et al., 2018), in which series of precipitation steps (calcium precipitation at alkaline pH and acid precipitation at 4 °C and 30 °C) were applied. The proposed process of the present study involved only the application of a physical separation and selective solubilisation of β-casein at low temperatures. A major advantage of the proposed process is the absence of added chemicals to facilitate the separation. Further research will focus on the manufacture of CoF fresh cheese from the β-casein-depleted micellar casein concentrate and its sensory properties.

Acknowledgements

The IGF Projects of the FEI were supported via AiF within the programme for promoting the Industrial Collective Research (IGF) of the German Ministry of Economic Affairs and Energy (BMWi), based on a resolution of the German Parliament. Projects AiF 19688 N and AiF 17126 N. The authors would like to thank to Fabian Heck, Birgit Greif, Luc Mertz, and Nabil Chaib at the University of Hohenheim for their analytical and technical assistance. Dr. Peter Gschwind and Prof. Dr. Kohlus are gratefully acknowledged for the preparation of microscopic images and for their helpful discussions.

References

Ardisson-Korat, A. V., & Rizvi, S. S. H. (2004). Vatless manufacturing of low-moisture part-skim mozzarella cheese from highly concentrated skim milk microfiltration retentates. *Journal of Dairy Science, 87*, 3601–3613.

Atamer, Z., Post, A. E., Schubert, T., Holder, A., Boom, R. M., & Hinrichs, J. (2017). Bovine β-casein: Isolation, properties and functionality. A review. *International Dairy Journal, 66*, 115–125.

Bachar, M., Mandelbaum, A., Portnaya, I., Perlstein, H., Even-Chen, S., Barenholz, Y., et al. (2012). Development and characterization of a novel drug nanocarrier for oral delivery, based on self-assembled β-casein micelles. *Journal of Controlled Release, 160*, 164–171.

Baum, F., Fedorova, M., Ebner, J., Hoffmann, R., & Pischetsrieder, M. (2013). Analysis of the endogenous peptide profile of milk: Identification of 248 mainly casein-derived peptides. *Journal of Proteome Research, 12*, 5447–5462.

Creamer, L. K., Berry, G. P., & Mills, O. E. (1977). A study of the dissociation of bcasein from the bovine casein micelle at low temperature. *New Zealand Journal of Dairy Science and Technology, 12*, 58–66.

Crowley, S. V., Caldeo, V., McCarthy, N. A., Fenelon, M. A., Kelly, A. L., & O'Mahony, J. A. (2015). Processing and protein-fractionation characteristics of different polymeric membranes during filtration of skim milk at refrigeration temperatures. *International Dairy Journal, 48*, 23–30.

DIN. (2005). *Milk and milk products - Determination of lactose and D-galactose content - Enzymatic method*. Berlin: Beuth Verlag GmbH.

DIN. (2013). *Lactose - Determination of water content - Karl Fischer method (ISO 12779:2011); German version EN ISO 12779:2013*. Berlin: Beuth Verlag GmbH.

Downey, W. K., & Murphy, R. F. (1970). The temperature-dependent dissociation of βcasein from bovine casein micelles and complexes. *Journal of Dairy Research, 37*, 361–372.

Famelart, M. H., & Surel, O. (1994). Caseinate at low temperatures: Calcium use in β-casein extraction by microfiltration. *Journal of Food Science, 59*, 548–553.

Flüeler, O., & Puhan, Z. (1977). Ultrafiltration in der Herstellung von Quark. *Deutsch Milchwirtschaft, 28*, 676–683.

Friis, T. (1981). Production of quarg by membrane filtration. *North European Dairy Journal, 47*, 165–170.

Hoffmann, W., Johannsen, N., & Stroebel, D. (2006). Anreicherung von Milcheiweißfraktionen durch Mikrofiltration [Enrichment of milk protein fractions via microfiltration]. *Kieler Milchwirtschaftliche Forschungsberichte, 58*, 41–51.

Huppertz, T., Hennebel, J.-B., Considine, T., Ur-Rehman, S., Kelly, A. L., & Fox, P. F. (2006). A method for the large-scale isolation of β-casein. *Food Chemistry, 99*, 45–50.

ISO. (2002). *Milk and milk products - determination of nitrogen content - routine method using combustion according to the Dumas principle (ISO 14891jIDF 185: 2002)*. Geneva, Switzerland: International Standardisation Organisation.

ISO. (2005). *Liquid milk - determination of acid-soluble beta-lactoglobulin content - reverse-phase HPLC method (ISO 13875jIDF 178:2005)*. Geneva, Switzerland: International Standardisation Organisation.

Kersten, M. (2001). *Proteinfraktionierung mittels Membrantrennverfahren [Protein fractionation by means of membrane processing]* (PhD Thesis). Munich, Germany: Technical University of Munich.

Konrad, G., Kleinschmidt, T., & Faber, W. (2012). Ultrafiltration flux of acid whey obtained by lactic acid fermentation. *International Dairy Journal, 22*, 73–77.

Körzendörfer, A., Nöbel, S., & Hinrichs, J. (2017). Particle formation induced by sonication during yogurt fermentation – impact of exopolysaccharide-producing starter cultures on physical properties. *Food Research International, 97*, 170–177.

Law, A. J. R., & Leaver, J. (2007). *Methods of extracting casein fractions from milk and caseinates and production of novel products*. WO patent 2003003847.

Lawrence, N. D., Kentish, S. E., O'Connor, A. J., Barber, A. R., & Stevens, G. W. (2008). Microfiltration of skim milk using polymeric membranes for casein concentrate manufacture. *Separation and Purification Technology, 60*, 237–244.

Le Berre, O., & Daufin, G. (1994). Microfiltration (0.1 μm) of milk: Effect of protein size and charge. *Journal of Dairy Research, 88*, 2–3.

Le Magnen, C., & Maugas, J.-J. (1995). *Method for obtaining beta-casein*. US Patent 5397577.

Lucey, J. A., Srinivasan, M., Singh, H., & Munro, P. A. (2000). Characterization of commercial and experimental sodium caseinates by multiangle laser light scattering and size-exclusion chromatography. *Journal of Agricultural and Food Chemistry, 48*, 1610–1616.

Maubois, J.-L., & Ollivier, G. (1992). *Milk protein fractionation. FIL-IDF Special Issue, 9201* (pp. 15–22).

McCarthy, N. A., Wijayanti, H. B., Crowley, S. V., O'Mahony, J. A., & Fenelon, M. A. (2017). Pilot-scale ceramic membrane filtration of skim milk for the production of a protein base ingredient for use in infant milk formula. *International Dairy Journal, 73*, 57–62.

Moitzi, C., Portnaya, I., Glatter, O., Ramon, O., & Danino, D. (2008). Effect of temperature on self-assembly of bovine β-casein above and below isoelectric pH. Structural analysis by cryogenic-transmission electron microscopy and small-angle X-ray scattering. *Langmuir, 24*, 3020–3029.

Murphy, J., & Fox, P. (1991). Fractionation of sodium caseinate by ultrafiltration. *Food Chemistry, 39*, 27–38.

O'Mahony, J. A., McSweeney, P. L. H., & Lucey, J. A. (2008). Observations on the rheological and functional properties of model cheeses made using milk protein concentrate solutions with different ratios of αs1-: β-Casein. *Milchwissenschaft, 63*, 145–148.

O'Mahony, J. A., Smith, K. E., & Lucey, J. A. (2007). *Purification of beta casein from milk*. US Patent, US 2007/0104847 A1.

Patel, R. S., Reuter, H., & Prokopek, D. (1986). Production of quarg by ultrafiltration. *Journal of the Society of Dairy Technology, 39*, 27–31.

Pierre, A., & Brule, G. (1981). Mineral and protein equilibria between the colloidal and soluble phases of milk at low temperature. *Journal of Dairy Research, 48*, 411–428.

Post, A. E., Ebert, M., & Hinrichs, J. (2009). β-casein as a bioactive precursor - processing for purification. *Australian Journal of Dairy Technology, 64*, 84–88.

Post, A. E., & Hinrichs, J. (2010). Suitability of commercial caseinates in comparison to micellar casein as raw material for isolation of food-grade β-casein. *Milchwissenschaft, 65*, 195–198.

Post, A. E., & Hinrichs, J. (2011). Large-scale isolation of food-grade β-casein. *Milchwissenschaft, 66*, 361–364.

Pouliot, M., Pouliot, Y., Britten, M., Maubois, J., Fauquant, J., & Maubois, J. (1994). Study of the dissociation of β-casein from native phosphocaseinate Study of the dissociation of β-casein from native phosphocaseinate. *Lait, 74*, 325–332.

Schäfer, J., Läufle, I., Schmidt, C., Atamer, Z., Nöbel, S., Sonne, A., et al. (2018). The sol-gel transition temperature of skim milk concentrated by microfiltration as affected by pH and protein content. *International Journal of Dairy Technology, 71*, 585–592.

Schäfer, J., Sebald, K., Dunkel, A., Hofmann, T., Rosenthal, I., Schuster, R., et al. (2019). A feasibility study on the pilot scale manufacture of fresh cheese from skim milk retentates without acid whey production : Effect of calcium content on bitterness and texture. *International Dairy Journal, 93*, 72–80.

Schubert, T., Meric, A., Boom, R., Hinrichs, J., & Atamer, Z. (2018). Application of a decanter centrifuge for casein fractionation on pilot scale: Effect of operational parameters on total solid, purity and yield in solid discharge. *International Dairy Journal, 84*, 6–14.

Sebald, K., Dunkel, A., Schäfer, J., Hinrichs, J., & Hofmann, T. (2018). Sensoproteomics: A new approach for the identification of taste-active peptides in fermented foods. *Journal of Agricultural and Food Chemistry, 66*, 11092–11104.

Seibel, J. R., Molitor, M. S., & Lucey, J. A. (2015). Properties of casein concentrates containing various levels of beta-casein. *International Journal of Dairy Technology, 68*, 24–29.

Shon, J., & Haque, Z. U. (2007). Functional attributes of native and thermized sour and sweet whey. *International Journal of Dairy Technology, 60*, 135–142.

Thienel, K. J. F., Holder, A., Schubert, T., Boom, R. M., Hinrichs, J., & Atamer, Z. (2018). Fractionation of milk proteins on pilot scale with particular focus on β-casein. *International Dairy Journal, 79*, 73–77.

van Hekken, D. L., & Holsinger, V. H. (2000). Use of cold microfiltration to produce unique β-casein enriched milk gels. *Lait, 80*, 69–76.

VDLUFA. (2011). *Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (Hrsg.): Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA- Methodenbuch), Bd. VI. Chemische, physikalische und mikrobiologische Untersuchungsverfahren für Milch, Milchprodukte und Molkereihilfsstoffe*. Darmstadt, Germany: VDLUFA Verlag.

Zulewska, J., Kowalik, J., & Dec, B. (2018). Flux and transmission of β-casein during cold microfiltration of skim milk subjected to different heat treatments. *Journal of Dairy Science, 101*, 10831–10843.