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RESEARCH ARTICLE

Temperature-controlled gelation of casein concentrates enabled by the utilisation of acid whey permeate as a diafiltration medium in microfiltration

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In this study, ultrafiltration permeate obtained from acid whey (AWP) was applied as a diafiltration (DF) medium during skim milk microfiltration. The thereby induced acidification of differently concentrated retentates was assessed regarding suitability to generate gels from micellar casein concentrates further downstream. While aggregation was avoided during cold DF, subsequent heating induced controlled spontaneous formation of gels with concentration- and pH-dependent firmness ranging from 26 to 1420 Pa. By employing its typical compounds, AWP as a rather problematic dairy side stream could thus be utilised for the production of calcium-rich acidic casein gels providing sensory characteristics of cultured dairy products.

Keywords Acidification, Casein, Coagulation, Diafiltration, Microfiltration, Milk gels, Whey.

INTRODUCTION

During the production of most acidified dairy products, a slow reduction of the pH below 4.8 by lactic acid fermentation at ~20–45°C induces the aggregation of casein micelles as the major building blocks of thereby derived coherent gel structures. The use of micellar casein concentrate (MCC) or milk protein concentrate as a base material for rennet or acid gelation gained considerable interest for producers due to potential economic benefits compared to the processing of standard milk (Xia *et al*. 2021). During the typically applied milk protein fractionation by microfiltration to obtain MCC from milk, lactose, minerals and whey proteins are washed out with the permeate stream in the so-called diafiltration (DF), where the serum phase is replaced by deionised water (Pierre *et al*. 1992; Xia *et al*. 2021). Downstream acidification of the obtained MCC by fermentation for gelation purposes is impaired by the increased buffering capacity of the greater amount of colloidal calcium phosphate within the concentrated casein fraction. This results in an extended fermentation duration and a bitter taste of derived products mainly related to thereby built peptides (Guinee *et al*. 2009; Sebald *et al*. 2018). Different authors applied a limited pH reduction *via* the addition of CO_2, HCl, glucono-δ-lactone, citric or lactic acid to reduce the buffering capacity of the concentrated casein fraction and enable a satisfactory subsequent fermentation (Brandsma and Rizvi 1999; Guinee *et al*. 2009; Eshpari *et al*. 2015; Schäfer *et al*. 2019; Gaber *et al*. 2020). As calcium is thereby transferred from the micellar to the serum phase, which is typically at least partially discarded, these approaches involve a reduction of the calcium content of the obtained products instead of keeping it as a valuable nutrient (Xia *et al*. 2021).

An alternative compound for pre-acidification during DF of MCC was the dairy side stream acid whey (AW) and especially protein-free ultrafiltration permeate obtained from it (AWP). These liquid co-products are derived from the production of different acidified dairy products like Greek-style yoghurt or fermented cheeses (Menchik *et al*. 2019). In comparison to the milk serum phase, they provide increased contents of lactate, calcium and phosphate, which are mostly already accompanied by aroma-relevant substances from prior fermentation (Cha *et al*. 2004; Menchik *et al*. 2019; Reitmaier and Kulozik 2022). Several approaches for the utilisation of these side streams have

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been described. However, due to efforts related to the relatively low pH (≤5) and high ionic load or requirement of specific technical equipment, these abundantly available side streams still often end up as animal feed or are disposed of causing costs for producers (Carvalho *et al*. 2013; Menchik *et al*. 2019; Pires *et al*. 2021). The application for DF could result in a more sustainable direct utilisation in dairy processing, meaning an added-value application of the contained valuable compounds (Pires *et al*. 2021).

During DF, the change in the serum phase induces a gradual alteration of the chemical environment towards the composition of the applied DF medium, which modifies the properties and interactions of the micellar casein phase. As casein mainly contributes to the formation of a deposit layer on the membrane surface, changes in casein micelles play a key role for overall process performance in terms of flux and transmission of solutes (Vetier *et al*. 1988; Bouzid *et al*. 2008; Reitmaier *et al*. 2021). A reduction of pH and an increase in the serum calcium content were both reported to negatively affect filtration performance due to intensified deposit formation by proteins at the membrane surface (Kessler *et al*. 1982; Vetier *et al*. 1988; Attia *et al*. 1991; Brandsma and Rizvi 1999; Rabiller-Baudry *et al*. 2005; Aaltonen 2013). An increased temperature was shown to foster casein aggregation at pH >5, especially if casein is in a concentrated state (Thomar and Nicolai 2016). Therefore, when applying AWP as DF medium, the resulting structural properties of the casein fraction and the deposit layer will be influenced by typical processing conditions such as temperature, casein concentration and the proportion of the serum phase being exchanged.

Based on this knowledge, we assumed that an also described alternative mechanism for the gelation of casein at reduced pH, which is referred to as the 'pH-T-route' (Vasbinder *et al*. 2003) could be applied for MCC when using of AWP in cold DF. In more detail, while avoiding instant aggregation during the addition of acidic compounds at low temperatures (direct acidification) linked to reduced hydrophobic interactions, a spontaneous formation of coherent gel structures from casein solutions can be induced by the application of a temperature increase (Roefs 1986; Vasbinder *et al*. 2003; Nguyen *et al*. 2016; Thomar and Nicolai 2016; Schäfer *et al*. 2018). This approach enables controlled spontaneous coagulation of casein and has therefore recently gained interest due to its potential applicability for the 3D printing of casein gels (Nöbel *et al*. 2021). Compared to fermentation with meso- or thermophilic cultures, this coagulation method could reduce processing time and related costs. However, the approaches described by abovementioned authors do not provide the typical aromatic profile of established acidic dairy products.

Whether the application of AWP during DF can result in alterations of casein micelles in the MCC enabling a suitable pre-acidification prior to downstream gelation or even an application of the pH-T-route, provided that the temperature during DF is kept low, has not been investigated yet. Therefore, the main objective of our study was to show that a typically applied cold filtration at ~10°C could enable controlled gelation of MCC through a temperature increase after DF with AWP. We hypothesised that by this novel approach, AWP could be applied as an efficient agent for the direct acidification of MCC also allowing for an adjustment of the properties of derived gels in a wide range through changes in typical processing variables. In contrast to filtration under chilled conditions, for 50–55°C as a further industrially applied temperature regime during MF/DF, an aggregation during DF with AWP can be expected. However, if a direct aggregation was not induced, a decrease in pH in MCC before rennet gelation in the sense of an often carried out pre-acidification could be achieved. In both cases, aroma compounds typically derived from fermentation could be included in obtained products without actually applying fermentation to the MCC. Besides investigations on the gelation of MCC after DF, gaining knowledge on the structural state of the casein micelles during filtration and the impact on fractionation performance is crucial when aiming at transferability of this approach to industrial application.

METHODS

Applied media

One batch of pasteurised (72°C, 30 s) skim milk (c_{Casein} = 29.17 g/L, $c_{\text{Whey protein}}$ = 5.22 g/L, c_{Calcium} = 1.30 g/L) from a local dairy (Molkerei Weihenstephan GmbH & Co. KG, Freising, Germany) was kept at 4°C until further application as feed material. Three different single filtration experiments and related analyses were carried out within 5 days to prevent microbial growth. One batch of industrially produced AWP, which had been obtained from acid curd cheese whey, was applied as main DF medium. Its pH of 4.65 and calcium concentration of 1140 mg/L lie within the typical and characteristic range reported for this type of dairy side stream (Menchik *et al*. 2019; Reitmaier and Kulozik 2022). As they coincide perfectly with compositional data of this distinct side stream derived from former sampling tests (n = 2), this detailed chemical characterisation is provided in Table 1. For analytical methods, see Reitmaier and Kulozik (2022).

Filtration setups

During crossflow microfiltration of 25 L skim milk at pilot scale, a 'constant-volume batch' DF was applied (Lipnizki *et al*. 2002). In this mode, one DF step represents a washing cycle with a DF medium in an amount that equals the starting feed volume. To maintain a constant fill level of the retentate during filtration, the supply with AWP was continuously adapted to the permeate volume flow. This induced a

Table 1 Analytical characterisation of acid whey permeate (AWP).

Analytical value	Mean ± SEM
pH 10°C	4.73 ± 0.03
pH 50°C	4.68 ± 0.02
Conductivity 10°C (S/m)	0.729 ± 0.074
Conductivity 50°C (S/m)	0.722 ± 0.082
Freezing point depression (m°C)	6331 ± 425
Dry matter (%)	5.65 ± 0.14
Sodium (g/L)	0.316 ± 0.052
Potassium (g/L)	1.483 ± 0.063
Magnesium (g/L)	0.158 ± 0.008
Calcium (g/L)	1.181 ± 0.035
Chloride (g/L)	1.311 ± 0.076
Phosphate (g/L)	1.762 ± 0.187
Citrate (g/L)	0.900 ± 0.008
Sulphate (g/L)	0.103 ± 0.018
Lactose (g/L)	37.89 ± 3.01
Lactate (g/L)	6.73 ± 0.77

SEM, standard error of the mean, $n = 2$.

gradual exchange of the serum phase surrounding the casein micelles and related ionic environment from native towards AWP composition. Kühnl *et al*. (2010) and Reitmaier *et al*. (2021) presented the temperature-controlled filtration rig used in our tests technically in detail. Filtration experiments were carried out according to Reitmaier *et al*. (2021) at a constant Δp_{TM} of 1×10^5 Pa and a wall shear stress of 150 Pa with a ceramic 23-channel membrane (ISOFLUX®, 0.14 µm, $A = 0.35$ m^2; TAMI Industries, Nyons, France). Cleaning, pre-conditioning with alkaline and washing with water before each trial were carried out according to Kühnl *et al*. (2010). We conducted two different experimental setups at 10°C, each comprising two DF steps with AWP (Figure 1). Individual processing steps are indicated by the abbreviation *CF* or DF followed by a digit representing the accomplished volume concentration factor of the retentate phase and the number of DF steps, respectively.

First, we tested whether gelation functionality can be achieved at different casein concentrations in the retentate after a change of the serum phase towards AWP (Experiment A). Therefore, DF was carried out in the context of microfiltration of skim milk prior to a concentration step to different concentration factors (*CF*) of *CF* 2, *CF* 3 and *CF* 4 through ultrafiltration (10 kDa, T-Series Omega® membranes, Pall Corporation, Port Washington, WI, USA). To test a potential benefit on the process performance of a concentration step before DF, which is typically conducted in industrial applications, the second experiment with a volume-related pre-concentration to *CF* 3 followed by DF with AWP was carried out (Experiment B). For these experiments, skim milk and AWP were adjusted to 10°C in a water bath. An additional microfiltration test without the application of a pre-concentration step was carried out at 50°C (Experiment C). Within this experiment, DF with AWP was followed by DF with deionised water as a filtration-enhancing medium to test the reversibility of potentially induced crosslinking effects in the deposited protein layer. Milk, AWP or deionised water were each stirred and pre-heated in a water bath for 15 min adjusted to 53 ± 1°C to avoid whey protein denaturation or salt precipitation.

To assess the major compositional changes induced during filtration experiments, the content of casein, major whey proteins, lactose and lactate in retentate samples was measured by RP-HPLC methods described in detail by Dumpler *et al*. (2016). Permeates were accordingly analysed for their concentration of β-casein and major whey proteins to enable evaluation of the separation efficiency. Agilent ChemStation software (Rev. B.04.03) was used for data analysis of RP-HPLC chromatograms. Filtrate mass flow was determined gravimetrically. From the membrane surface area (A), measured permeate mass (dm) and time (dt) differences, the mass-based flux (J) could be calculated by Eqn (1).

$$J = \frac{1}{A} \bullet \frac{dm}{dt} \quad (1)$$

To detect the most relevant changes in the ionic environment over DF progress, the pH of the retentate was monitored and the calcium content of the retentate/permeate phases was investigated by flame photometry (ELEX 6361; Eppendorf AG, Hamburg, Germany). We calculated the micellar calcium content ($c_{Ca\ Mic}$) of the different samples from the overall concentration present in the retentates ($c_{Ca\ Ret}$) and the serum phase concentration deduced from analysis of the corresponding permeate samples ($c_{Ca\ Per}$) (Reitmaier *et al*. 2021). To account for the concentration of casein present in the corresponding retentate, the application of different excluded volume factors (VF) was applied according to Ferrer *et al*. (2011).

$$c_{Ca\ Mic} = c_{Ca\ Ret} - c_{Ca\ Per} * \mathrm{VF} \quad (2)$$

The average particle size of casein micelles was determined using static light scattering. This was carried out to detect possibly induced undesired aggregation of casein micelles in the retentate phases inside the filtration rig prior to gel formation by temperature increase. $d_{50,3}$ was calculated based on the Mie theory from measurements of the particle size distribution within the range of 0.02–2000 µm by the internal software of a Mastersizer 2000 unit (Malvern Instruments GmbH, Herrenberg, Germany). Samples were diluted in water in a Malvern Hydro 2000S dispersion device (Malvern Instruments GmbH).

Investigations on gelation functionality of MCC after filtration

Rheological recording (MCR302, Geometry CP-50-2; Anton Paar, Graz, Austria) of the viscoelastic properties served to

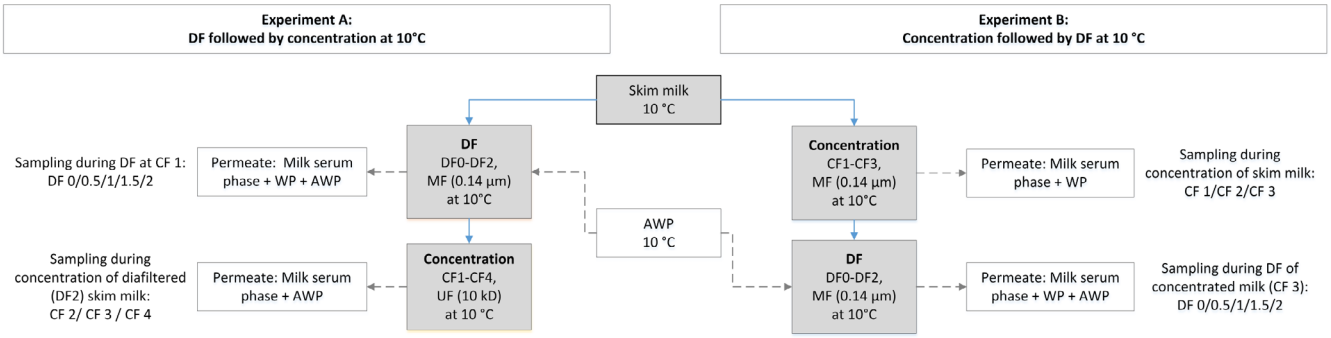

Figure 1 Trial setup employing diafiltration (DF) with acid whey permeate (AWP) before (experiment A) or after (experiment B) concentration at 10°C.

detect potential acid-heat gelation of the differently diafiltered and concentrated retentate samples obtained from filtrations at 10°C. Temperature ramps following the scheme 10–80–10°C with 600 s residence time of each step were carried out while avoiding drying off during the measurement by a steam trap. Elastic (G′) and loss moduli (G″) were monitored every 20 s at a frequency of 0.1 Hz and a strain of 0.01 to meet small-amplitude oscillatory shear conditions (Gunasekaran and Ak 2000).

Statistical analysis

Data were plotted with Sigmaplot 13 (Systat Software Inc., San Jose, CA, USA). The majority of the presented values was derived from single measurements. Besides the mean, the standard error of the mean indicates the actually measured values and the span in the case of $n = 2$.

RESULTS AND DISCUSSION

Application of AWP in pilot scale DF at 10°C and tests on gelation functionality

We performed filtration experiments with AWP as DF medium at 10°C to assess the effects of low pH on filtration performance and micellar properties. The change from native milk serum to AWP during DF should be accompanied by an approximately 3-fold increase in the calcium content in the serum phase surrounding the casein micelles (~350–400 mg/L in milk). As ionic calcium is counterbalanced by phosphate compounds, this calcium enrichment may not be linked to a major increase in calcium activity, which could nevertheless also foster micellar interaction (Dumpler *et al*. 2017). The undesired immediate aggregation, however, should be suppressed during low-temperature filtration. A controlled increase in temperature in a rheometer subsequent to DF was applied to test the kinetics of gelation of casein retentates and to assess the developing gel strength for different degrees of environmental exchange. As we considered the casein concentration during or after the exchange of the ionic environment by DF with AWP to be decisive for the formation of a gel, two separate experiments were carried out: One with a concentration step before and one after DF. Pre-concentration by microfiltration before DF to *CF* 3 or concentration after MF/DF to *CF* 4 employing ultrafiltration was carried out (Figure 1). DF was stopped after two DF steps when an exchange of the serum phase of theoretically 86% was accomplished (Reitmaier *et al*. 2021). The decrease in lactose content and increase in lactate concentration (Tables 2 and 3) towards the composition of AWP (Table 1) reflect this progressive exchange along DF with AWP in both experiments A and B. The protein composition of the obtained retentate and permeate samples presented in Tables 2 and 3 reveal that only during the microfiltration steps carried out to increase the casein content prior to starting DF in experiment A, a relevant transmission of β-casein accompanied the transmission of whey proteins. This well-known phenomenon is related to the release of β-casein from the casein micelle at a temperature at or below 10°C in monomeric form into the milk serum, which could be minimised by different means (Schiffer *et al*. 2021a; 2021b) or utilised for casein fractionation (Schäfer *et al*. 2019). The environmental exchange during AWP-DF resulted in reduced transmission of β-casein as well as whey proteins as also indicated by the nearly stable level of residual whey proteins after one DF step in both trials (Figures 2a,b). As a consequence, the casein content of retentate samples obtained at *CF* 2 and *CF* 3 was lower in experiment B (pre-concentration) than in experiment A (concentration after DF). However, the casein/whey protein ratio in the retentates continuously increased in both cases during microfiltration (Tables 2 and 3). The flux decreased progressively over the course of DF in both filtration setups at 10°C (Figure 2a,b). A flux reduction of 49% was observed after two DF steps. A similar behaviour regarding filtration performance and separation efficiency has been reported by Brandsma and Rizvi (1999) and Schäfer *et al*. (2019) during skim milk microfiltration at reduced pH.

When DF was carried out at *CF* 3, the concentrated state resulted in a lower flux level, which can be attributed to a

Table 2 Compositional data of retentate and permeate samples obtained during filtration experiment A: DF (microfiltration) against AWP at *CF* 1 followed by concentration (ultrafiltration) at 10°C.

Process stage		Feed/Retentate					Permeate	
CF	DF step	$c_{Lactose}$ (g/L)	$c_{Lactate}$ (g/L)	c_{Cas} (g/L)	c_{WP} (g/L)	Cas:WP	$c_{\beta\text{-}Cas}$ (g/L)	c_{WP} (g/L)
(1)	0	43.1	0.1	29.16	5.02	5.81	0.21	1.60
(1)	0.5	45.1	2.3	28.64	4.52	6.34	0.09	1.00
(1)	1	42.2	3.9	27.78	4.15	6.69	0.07	0.77
(1)	1.5	42.1	4.4	28.19	3.99	7.07	0.04	0.63
(1)	2	39.6	5.3	27.97	3.87	7.23	0.03	0.35
2	(2)	39.3	6.8	56.39	7.78	7.24	–	–
3	(2)	42.5	6.5	94.73	13.15	7.20	–	–
4	(2)	41.0	5.7	129.37	17.95	7.21	–	–

CF, Concentration factor; DF, diafiltration; DF step, one volume turnover relative to the feed volume by replacing the removed permeate by the DF medium; AWP, acid whey permeate; Cas, casein; WP, whey proteins.

Table 3 Compositional data of retentate and permeate samples obtained during filtration experiment B: pre-concentration (microfiltration) to *CF* 3 followed by DF (microfiltration) against AWP at 10°C.

Process stage		Retentate					Permeate	
CF	DF step	$c_{Lactose}$ (g/L)	$c_{Lactate}$ (g/L)	c_{Cas} (g/L)	c_{WP} (g/L)	Cas:WP	$c_{\beta\text{-}Cas}$ (g/L)	c_{WP} (g/L)
1	(0)	43.1	0.0	30.11	5.24	5.75	0.23	2.27
2	(0)	47.7	0.1	49.44	7.56	6.54	0.29	2.06
3	(0)	49.1	0.2	69.61	10.16	6.85	0.48	1.88
(3)	0.5	46.0	2.3	68.10	9.39	7.25	0.06	1.16
(3)	1	43.1	3.3	66.19	8.75	7.56	0.06	1.02
(3)	1.5	41.8	4.7	68.96	8.75	7.88	0.04	0.90
(3)	2	39.3	5.5	66.75	8.27	8.07	0.03	0.86

CF, Concentration factor; DF, diafiltration; DF step, one volume turnover relative to the feed volume by replacing the removed permeate by the DF medium; AWP, acid whey permeate; Cas, casein; WP, whey proteins.

higher viscosity and the increased casein content in the retentate. However, a relative flux reduction of only 15% was observed during the two consecutive DF steps. Further investigations on the calcium distribution, pH change and particle size of the retentate aimed at elucidating the mechanisms behind these observations. The changes in calcium concentration of the whole retentate, permeate/serum and the obtained micellar phase during DF with AWP at 10°C are presented in Figure 3(a,b) for *CF* 1 and *CF* 3, respectively. During the concentration steps, the content of calcium increased in both cases due to the accumulation of the micellar phase in the retentate, which is rich in calcium bound to the casein micelle structure. In contrast, the permeate/serum concentration remained at a stable level during the concentration steps. It can furthermore be seen for both filtration experiments that during DF the total and serum calcium content increased due to the high amount present in AWP as the DF medium. However, the difference between serum and micellar phases decreased during DF at *CF* 1, which shows that calcium was released from the micellar structure due to the reduced pH. No change in the particle size could be detected for *CF* 1 (Figure 4a) and for *CF* 3 (Figure 4b), although a continuous pH reduction to 5.5 and 5.8, respectively, was achieved. The lower pH reduction at *CF* 3 is related to the higher amount of casein micelles accounting for an enhanced buffering capacity of the retentate, which is also reflected by the nearly stable level of micellar calcium during DF (Figure 3b). This also explains the higher relative reduction of flux for the *CF* 1 experiment when compared to DF at *CF* 3. Increased interactions of proteins, which are physically forced to close distance in the deposited layer at the membrane surface, have most likely led to the creation of a more compact deposit. This explanation is supported by findings of Steinhauer *et al*. (2015), who reported on a decrease in deposit hydration as well as a larger share of the deposited casein mass in a gel-like state when reducing the pH of isolated casein micelles prior to filtration. A change in electrostatic protein interactions due to the reduction of pH is known to markedly contribute to increased deposit resistance R_D (Rabiller-Baudry *et al*. 2005). According to Aaltonen (2013), increased interactions of whey proteins should furthermore be considered a possible cause for flux reduction observed during filtration of milk at reduced pH, which is in line with observations of Steinhauer *et al*. (2016) on the pH dependence of zeta-potential, colloidal interactions and fouling resistance in ultrafiltration experiments with different isolated whey proteins. Besides pH-dependent interactions of the proteins, the calcium level of the serum was shown to be decisive in deposit structuration as it can act as an intra-micellar or

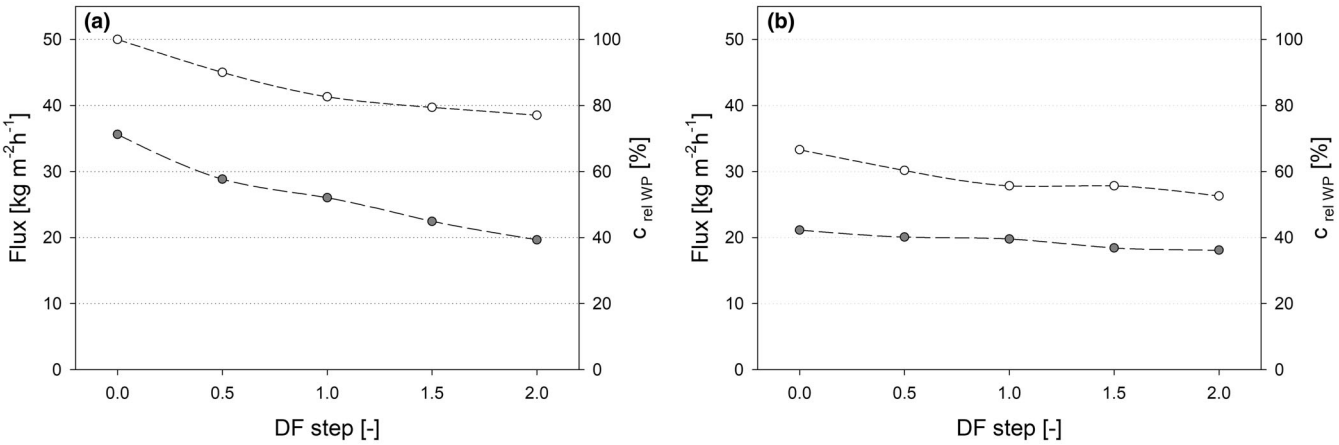

Figure 2 Evolution of flux (●) and relative residual concentration of WP in the retentate (○) over the progress of DF with acid whey permeate (AWP) at CF1 (a) and CF3 (b), 10°C.

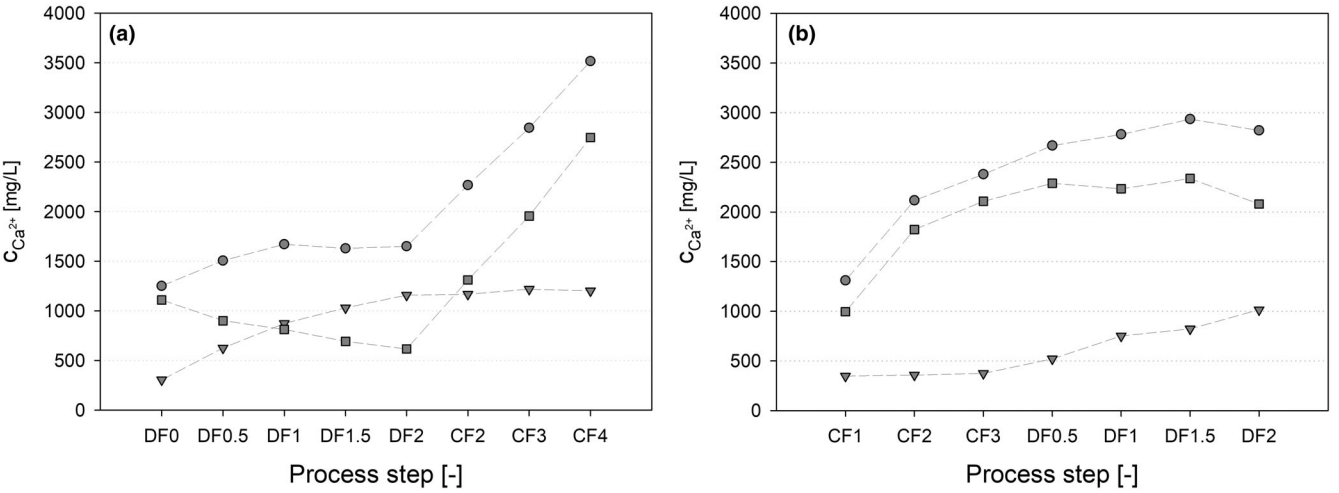

Figure 3 Evolution of calcium concentration in retentates (●) and permeates (▼) as well as micellar calcium (■) over the progress of diafiltration (DF) with acid whey permeate (AWP) at 10°C and subsequent concentration (a), respectively, after pre-concentration (b).

inter-micellar linking agent as well as between proteins and the membrane surface (Vetier *et al*. 1988; Attia *et al*. 1991; Kulozik 1998; Rabiller-Baudry *et al*. 2009; Reitmaier *et al*. 2021).

In summary, these factors lead to enhanced deposit formation and limit the use of AWP as a DF medium for efficient milk protein fractionation by microfiltration. Consequently, DF with AWP aiming at the further investigated gelation functionality should rather be applied in a finalising step of DF following standard concentration or fractionation operations of skim milk microfiltration. Also, this sequence of applying DF media, starting with water and applying AWP only during the very last DF stage, would ensure obtaining the whey protein fraction in a non-acidic environment.

Investigation on heat-induced gelation of MCC obtained from low-temperature DF with AWP

Rheometric tests involving the heating of retentate samples derived from DF trials aimed at testing the pH-T-gelation route for concentrated casein after a serum phase exchange towards AWP, which could be of scientific and also of practical interest. The creation of gel structures from these samples would result in a direct utilisation of lactic acid in AWP as an acidulant for the gelation of casein in a concentrated state also entrapping calcium and aromatic compounds as nutritionally and sensory relevant components. We considered a temperature ramp of 10°C → 80°C → 10°C suitable for gel formation as it reflects the typical temperature profile for the manufacture of Paneer, Chhana or Queso Blanco as traditional acid-heat induced cheeses. In

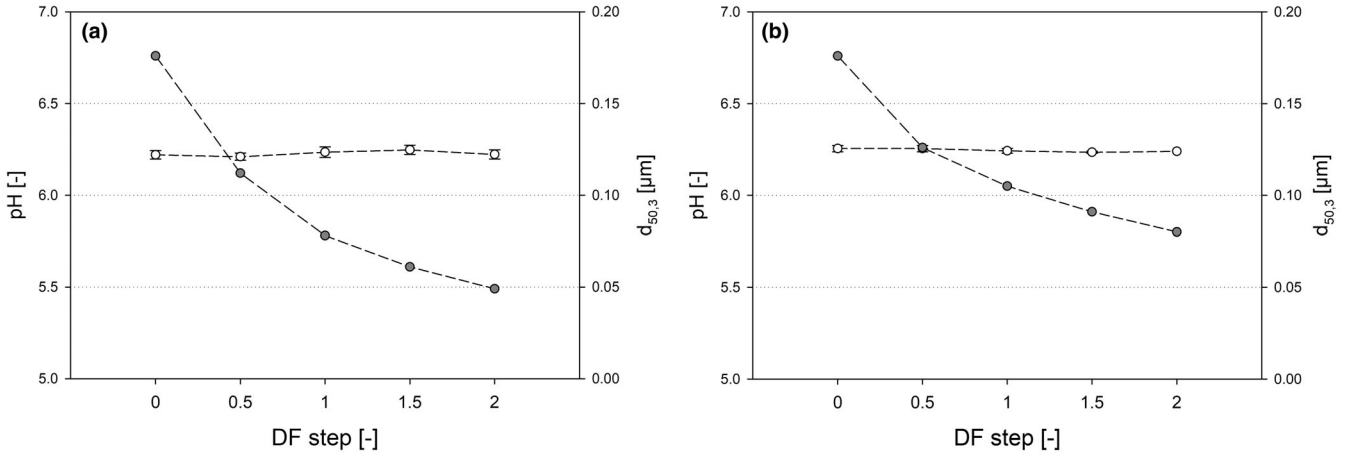

Figure 4 Evolution of pH (●) and $d_{50,3}$ (○) over the progress of diafiltration (DF) with acid whey permeate (AWP) at 10°C and *CF* 1 (a), respectively, *CF* 3 (b).

contrast to our pH-T approach, these types of cheese are obtained by instant coagulation of casein from buffalo's or cow's milk by the addition of different acidic compounds at elevated temperatures (Chandan 1991; Lucey 2011).

Figure 5 shows the storage modulus G' during a temperature sweep for retentate samples taken from the experiment with DF with AWP prior to retentate concentration. Figure 6 depicts the data for samples taken from the experiment where DF with AWP was carried out after a pre-concentration step to *CF* 3. The final level of G′ reflects the extent of elasticity of the sample, which is related to the achieved gel strength. Higher gel strength was achieved when increasing the degree of environmental exchange and casein concentration in both sets of experiments. More specifically, gelation was induced at a pH decrease to at least pH 5.8. For the samples concentrated after the DF at *CF* 1 by ultrafiltration, it can be seen in Figure 5 that for the sample obtained at *CF* 3, surprisingly a lower final level of G′ was reached than for the sample taken at *CF* 2. This could result from a slight increase in G′ followed by a decrease at around 50°C, which was observed for the *CF* 3 sample, but not at *CF* 2. This pre-gelation could be related to a connection of whey proteins and casein monomers as described by Lam *et al*. (2019), which disturbs the subsequently coagulating main gel structure with casein micelles as the primary building blocks. As a consequence, the G' profiles of the samples that were obtained under comparable conditions, namely after two DF steps at *CF* 3, developed differently in the two different experimental approaches (Figures 6 and 7). Due to the lower content of whey proteins and of β-casein, which were more effectively washed out during concentration prior to DF than after DF (Table 2), the described pre-gelation did not emerge for samples derived from experimental setup B and consequently, a higher final G' was achieved. The contribution of the different amounts of whey proteins (Tables 2 and 3), types of

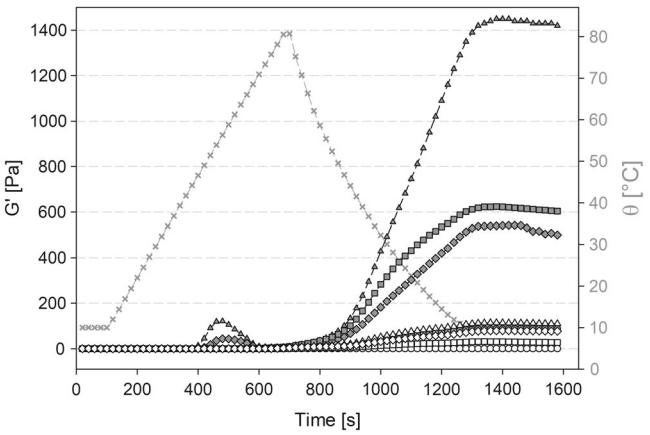

Figure 5 Evolution of G′ over time during temperature sweep (crosses, right *y*-axis) meant to induce heat gelation of samples derived from different stages of diafiltration (DF; 0–2 DF steps at *CF* 1) with acid whey permeate (AWP) and subsequent concentration (*CF* 1–4 after two-step DF) at 10°C (Setup A). CF1 after 0 DF steps (○); CF1 after 0.5 DF steps (□); CF1 after 1 DF step (◇); CF1 after 1.5 DF steps (△); CF1 after 2 DF steps (●); CF2 after DF (■); CF3 after DF (◆); CF4 after DF (▲).

interactions as well as alterations of the physicochemical state of calcium (Figure 3) to the observed formation of differently structured gels could be of scientific interest. Knowledge of these relations would enable a deeper mechanistic understanding of the coagulation approach presented in our study and could be subject to follow-up investigations employing advanced analytical setups.

To our knowledge, a similar setup for the casein gelation induced within our trials has so far only been described by Maubois and Kosikowski (1978), who put AW powder into milk before concentration by ultrafiltration and subsequent heating for the whey-less production of a ricotta-like cheese. However, our findings substantially broaden the applicability

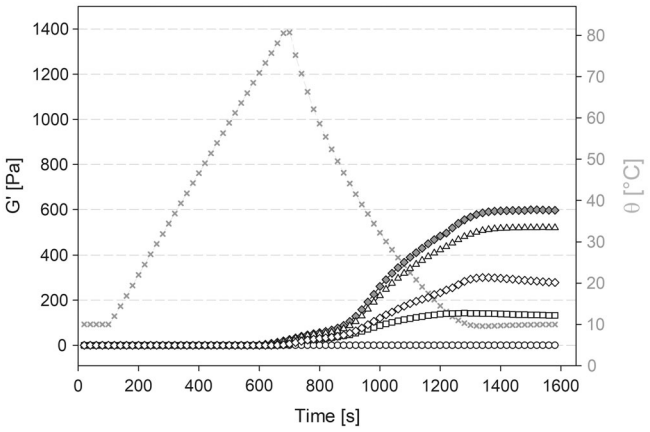

Figure 6 Evolution of G′ over time during temperature sweep (crosses, right y-axis) meant to induce heat gelation of pre-concentrated samples derived from different stages of diafiltration (DF) with acid whey permeate (AWP) after pre-concentration (0–2 DF steps at *CF* 3) (Setup B). *CF* 3 after 0 DF steps (○); *CF* 3 after 0.5 DF steps (□); *CF* 3 after 1 DF step (◇); *CF* 3 after 1.5 DF steps (△); *CF* 3 after 2 DF steps (◆).

of this gelation mechanism, making use of AWP as a direct acidulant in different ways. The direct use of AW or AWP for structure formation in concentrated casein solutions is also conceivable on an industrial scale. Figure 7 depicts a possible large-scale processing setup employing pre-concentration combined with DF followed by heating as major unit operations. As suggested by our results, the exchange of the serum phase of skim milk against AWP, which is typically obtained from a prior acid gel production involving fermentation, should be carried out subsequent to a pre-concentration step combined with DF with deionised water or ultrafiltration permeate from milk. At controlled temperature conditions, AWP could then be applied as DF medium generating acidified gels from concentrated casein after obtaining the so-called 'ideal whey' which contains whey proteins in their native state. This could overcome the disadvantage of a reduced filtration speed and poor depletion of whey proteins, which have also been described as major drawbacks of alternative pre-acidification approaches involving a filtration of MCC at reduced pH (Xia *et al*. 2021). However, for acid-induced and heat-induced casein gels, a positive influence of whey proteins on firmness has been reported (Dannenberg and Kessler 1988; Silva *et al*. 2018). Therefore, a variation in the content of whey proteins could besides the extent of environmental exchange before gelation or temperature adjustment lead to structural alterations of gel products derived from differently concentrated casein. As ceramic membranes are typically applied at 50–55°C rather than at 10°C in dairy practice, the use of polymeric membranes may also be considered for industrial implementation. Due to a continuous exchange of the serum phase during DF, some acidic effluent will be generated during the pre-acidification of MCC through DF with AWP. However, the partial binding of typical components of the utilised AWP within the retentate and gel formed therefrom will in comparison to alternative approaches not increase, but reduce the load of environmentally and procedural problematic substances of the rather unwanted coproduct AW (Schäfer *et al*. 2018; Xia *et al*. 2021). This entrapment is accompanied by an increase in the calcium content and other beneficial nutritive or aromatic compounds in the obtained gel (Cha *et al*. 2004). In sum, this approach offers several benefits in comparison to the application of the pH-T route by the addition of other acidulants and/or fermentation, which may however be better manageable in technical practice. In laboratory tests prior to the presented experimental setup, potential alternative options for an exchange of the ionic environment towards AWP by dialysis or mixing of AWP with

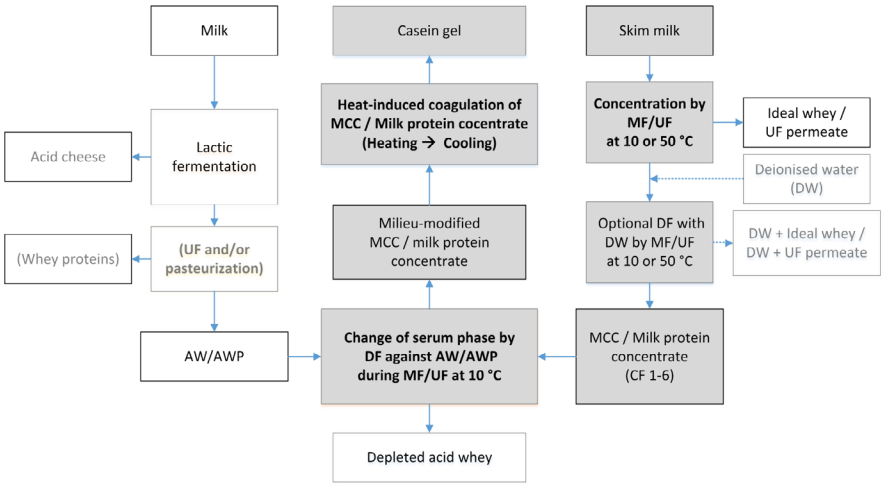

Figure 7 Possible processing scheme on an industrial scale with obtainable products involving the production of acid whey permeate (AWP) and MCC.

MCC also resulted in the gelation of casein at ≥50°C. However, these approaches are less practicable in our view or involve dilution of the prior concentrated casein, respectively.

Application of AWP at pilot scale DF at 50°C

A filtration experiment with AWP as DF medium (DF step 0–1) and subsequent washing with deionised water (DF step 1–2) was carried out at 50°C. This aimed at testing the expectation of a further enhanced deposit formation, even at low Δp_{TM}, due to increased hydrophobic forces during environmental exchange towards AWP at elevated temperature and possible irreversible compaction or aggregation. Table 4 provides analytical data on the major components of the retentates obtained over DF progress. Whereas the increase in the lactate and a slight reduction of the lactose concentration during DF step 1 indicate an exchange of the serum phase in direction of AWP, the decrease in both values during DF step 2 reflects the washout of components with deionised water. The serum exchange towards AWP during DF step 1 at 50°C was accompanied by a rapid decrease in the flux to 49% of the starting level after half a DF step, and to 41% after one full DF step, respectively. In parallel, we observed a reduced decrease in the residual content of whey proteins after 0.5 DF steps (Figure 8a), indicating a strong decrease in whey protein transmission, which is also reflected by protein-related data in Table 4. Compared to the results of DF at *CF* 1 at 10°C (Figure 2a), the flux decreased faster, but a similar reduction of 49% was measured after 2 DF steps. Similar compaction of the deposit seems to have occurred in both DF setups, which developed nearly instantly at 50°C in contrast to the gradually progressive formation that had been observed at 10°C. Upon applying deionised water in DF step 2 to test whether the intensified fouling induced by DF with AWP could be reversed, a slight further reduction of flux was observed, while whey protein transmission stabilised at the reduced level. This indicates the irreversibility of deposit compaction with markedly enhanced interactions resulting from reduced repulsion of casein micelles resulting from DF with AWP. Further investigations on casein micelles revealed that the calcium level in the retentate increased during the exchange of the ionic environment by AWP, which is also reflected by the calcium increase in the permeate (Figure 8b). The micelle-bound calcium was found to decrease due to the pH reduction (Figure 9), despite the already high calcium level in AWP. A noteworthy observation in this experiment at 50°C was the formation of relatively small casein aggregates, visible to the naked eye, already after the completion of only half of a DF step. Due to this rapid aggregation of

Table 4 Compositional data of retentate and permeate samples obtained during filtration experiment C: One DF step against AWP at *CF* 1 followed by one DF step against deionised water at 50°C.

Process stage		Retentate					Permeate	
CF	DF step	$c_{Lactose}$ (g/L)	$c_{Lactate}$ (g/L)	c_{Cas} (g/L)	c_{WP} (g/L)	Cas: WP	$c_{\beta\text{-Cas}}$ (g/L)	c_{WP} (g/L)
(1)	0	43.9	0.0	29.17	5.21	5.81	0.08	1.78
(1)	0.5	43.9	3.0	26.64	3.83	6.34	0.06	1.52
(1)	1	41.4	5.0	27.89	3.49	6.69	0.05	1.06
(1)	1.5	27.6	2.9	26.87	3.15	7.07	0.04	0.82
(1)	2	18.3	2.2	25.83	2.78	7.23	0.03	1.13

CF, Concentration factor; DF, diafiltration; DF step, one volume turnover relative to the feed volume by replacing the removed permeate by the DF medium; AWP, acid whey permeate, Cas, casein; WP, whey proteins.

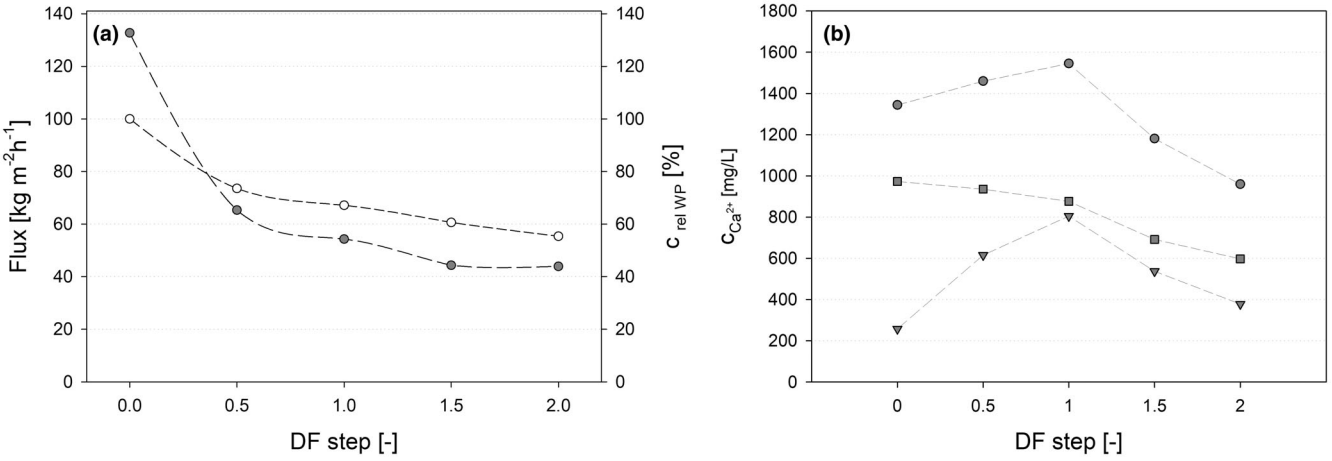

Figure 8 Evolution of flux (●), relative residual concentration of WP in the retentate (○) (a) as well as evolution of calcium concentration in retentates (●), permeates (▼) and micellar calcium (■) (b) along progress of one diafiltration (DF) step with acid whey permeate (AWP; DF step 0–1) followed by one DF step with deionised water (DF step 1–2) at 50°C.

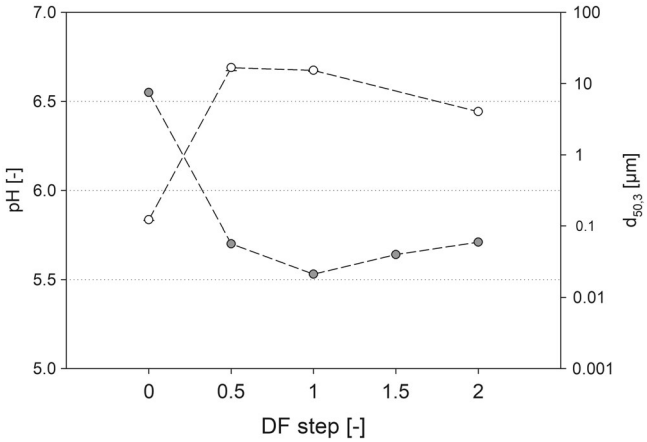

Figure 9 Evolution of pH (●) and $d_{50,3}$ (○) over the progress of diafiltration (DF) with acid whey permeate (AWP) (DF step 0→1) followed by DF with deionised water (DF step 1→2) at 50°C (A).

casein, DF with AWP at an elevated temperature of 50°C was shown to be not suitable for executing a limited acidification of MCC prior to further coagulation to coherent structures. These changes were also monitored by measuring the particle size of the casein retentates during DF (Figure 9). For the casein fraction, the shear applied during the continuous pumping of the retentate resulted in the formation of flocculated and suspended casein aggregates instead of a connected curd mass. In theory, these larger particles could be more efficiently removed from the membrane surface by the hydrodynamic forces under crossflow conditions, since their size is above the so-called critical diameter for particle erosion of only some micrometres (Ripperger and Altmann 2002). However, Le Berre and Daufin (1998) observed an increased filtration resistance for milk containing aggregated casein, which was also the case in our experiment. These authors hypothesised a resistance-enhancing deposition of smaller particles instead of casein micelles. In contrast to this explanation, Attia *et al*. (1991) and Pignon *et al*. (2004) observed *via* microstructural analyses the compaction and aggregation of casein micelles at reduced pH and, in turn, the formation of denser deposits on membranes fouled with acidified milk. Furthermore, precipitation of calcium salts was considered possible at elevated temperatures by some authors (Vetier *et al*. 1988; Rabiller-Baudry *et al*. 2009). If this non-protein-related scaling would have considerably contributed to the increased deposit formation in our experiment, the downstream ion washout during DF with deionised water should have induced a decrease in the deposit resistance R_D. This, however, was not the case.

The observed irreversibility upon further change of the ionic environment seems to be in contrast to the findings of St-Gelais *et al*. (1992), who reported a flux increase during DF with deionised water after filtration of acidified milk at reduced permeate flow. However, in their studies, the retentate was five-fold diluted with deionised water and the deposit was relaxed by stopping the filtration system prior to DF, which both may have contributed to a physical loosening of the compacted deposit. Our results demonstrate that under chemical environment conditions, which are more severe than changes of pH and calcium content applied by Reitmaier and Kulozik (2022), an irreversibly compacted state of the deposit was induced. Similar behaviour is well known for casein micelles in a native ionic environment in response to the application of a higher Δp_{TM} and related physical compaction (Gesan-Guiziou *et al*. 1999).

CONCLUSIONS

The applicability of AWP as DF medium and, at the same time, as a direct acidulant for controlled spontaneous casein gelation in various processing setups was tested in this study to open prospects of AWP utilisation. The application of AWP in this regard can be considered as a promising option for AWP valorisation to obtain mild acidic dairy products with a natural calcium content and a typical aromatic profile of cultured dairy products. The application would be limited to concentrated casein solutions that have to be heated after an exchange of the ionic environment at low temperature or gelled during the final DF stages at elevated temperature. A targeted adjustment of acid gel properties can be achieved by variation of the casein concentration, the speed of environmental exchange and choice of temperature profile from low temperature to the gelling temperature. The observed spontaneous gel formation upon temperature increase of low pH protein concentrates could furthermore provide an option for 3D-printing of milk protein gels, where fast gelling kinetics are required. A supportive role of the investigated application of AWP as a direct acidulant replacing pre-ripening by starter cultures especially prior to rennet cheese production without further addition of $CaCl_2$ could also be considered. For practical and scientific reasons, the impact of the whey protein content of applied casein concentrates on gels obtained by our approach and the possibility of a fat addition prior to gelation should be the subject of future investigations.

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AUTHOR CONTRIBUTIONS

Michael Reitmaier: Conceptualization; data curation; formal analysis; investigation; methodology; software; visualization; writing – original draft. **Ulrich Kulozik:**

Conceptualization; project administration; resources; supervision; validation; writing – review and editing.

CONFLICT OF INTEREST

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DATA AVAILABILITY STATEMENT

Research data are not shared.

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