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RESEARCH ARTICLE

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Compositional analysis of dairy side streams and assessment of their applicability as diafiltration media

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The direct utilisation of side streams from membrane processing and evaporation is of great potential interest for dairies to reduce water consumption. In an analytical screening of 89 industrially obtained processing side streams, samples varied in pH and conductivity in broad ranges of 4.22 to 7.56 and 0.001 to 0.949 Siemens per meter, respectively. Detailed compositional data of the side streams enabled an assessment of their applicability as diafiltration (DF) media for milk protein purification during microfiltration (MF) or ultrafiltration (UF). Microfiltration experiments with simulated nanofiltration (NF) permeates showed that slight compositional variations of DF media can significantly affect milk filtration performance.

Keywords Casein, Dairy effluent, Whey, Membrane processes, Microfiltration, Ultrafiltration.

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INTRODUCTION

Dairy processing operations produce aqueous side streams more or less close to water in different compositions, for example evaporation condensates, permeates from reverse osmosis, nano- and ultrafiltration. The reuse and valorisation of such streams accumulating in dairy production is one option to increase the sustainability of overall processing and to ensure future market competitiveness of dairies. Current approaches of the dairy sector mainly aim at the reuse of such side streams as purified water to reduce freshwater consumption and effluent load. However, the applied different technological set-ups to achieve the required purity also entail additional processing effort and energy consumption (Van Asselt and Weeks 2013; Galvão 2018; Chamberland *et al*. 2020; Pires *et al*. 2021). The direct utilisation of aqueous side streams, for example diafiltration (DF) medium during microfiltration (MF) or ultrafiltration (UF) of skim milk to obtain the proteins in fractionated form, could result in a more sustainable state of processing (Van Asselt and Weeks 2013). Within these standard dairy unit operations, smaller constituents like lactose and, in the case of MF, whey proteins are washed out in a DF process to obtain milk protein isolates (total milk protein or fractionated whey proteins and micellar casein) (De Boer 2014). During cross-flow microfiltration in DF mode aiming at mitigating membrane fouling by the retained fraction, minor components are gradually transferred by the aqueous phase into the permeate. Diafiltration requires the use of large volumes of a washing/DF medium to achieve the typically desired high purity of the retained protein phase (Tomasula and Bonnaillie 2015; Chamberland *et al*. 2020). Therefore, this industrial process offers a high potential for the direct reuse of available dairy side streams; however, their applicability will highly depend on their chemical composition. Deionised water or reverse osmosis (RO) permeate of not clearly stated origin is described to be typically used as DF media to remove lactose and serum minerals, primarily Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^- and phosphate and citrate species (Gaucheron 2011; De Boer 2014). The related gradual modification of pH and ionic strength of the serum phase affects the colloidal state of the milk proteins (Kulozik and Kersten 2002). Several authors reported on an increase in filtration performance due to changes of the deposited layer structure at the membrane surface during DF of milk with deionised water (Adams and Barbano 2013; Liu *et al*. 2017; Ng *et al*. 2018; Reitmaier *et al*. 2021).

This layer generates significant resistance against mass transport towards and through the membrane pores, which impairs filtrate permeation (flux) and the transmission of solutes. In particular, changes in electrostatic interaction between casein micelles and their hydration are known to affect the structure of this deposited layer and the related impact as a secondary membrane in MF of milk (Vetier *et al*. 1988; Bouzid *et al*. 2008; Reitmaier *et al*. 2021). Other studies revealed an altered functionality of the casein fraction after the typically applied DF with deionised water. These changes were related to structural changes of casein micelles induced by the modified milieu in conjunction with the major ion washout, particularly calcium as a structure-relevant component, and pH shift (Ferrer *et al*. 2014; Eshpari *et al*. 2015; Reitmaier *et al*. 2021). Ultrafiltration permeate obtained from skim milk can be applied as DF medium to avoid such changes of the milk milieu by preserving the native structure of casein micelles (Kulozik and Kersten 2002). However, this DF medium will not allow for a washout of lactose, which limits the protein content and the purity of the obtained product.

Despite possible regional legislative limitations, several abundantly available dairy side streams could be potential alternative candidates for an application in protein purification by means of DF. The chemical composition of these media depends on the type of dairy liquid used as source material (milk, sweet or acid whey), the process set-up and the operating conditions. Especially, vapour condensates, nanofiltration (NF) and UF permeates or thermo-curd whey obtained during centrifugation of acid cheese varieties could be potential candidates due to a principal absence of proteins. Over time along a DF process and depending on the use of different aqueous media, a significant compositional variation will emerge in industrial practice. Literature data on the composition of aqueous side streams are either limited to the sampling of single locally available sources of mostly unknown origin or focused on pollutant load parameters relevant for effluent treatment or land use (Mavrov *et al*. 2001; Wojdalski *et al*. 2013; Gami *et al*. 2016; Slavov 2017; Ahmad *et al*. 2019; Oliveira *et al*. 2019; Rasmussen *et al*. 2020; Merkel *et al*. 2021).

Therefore, the aim of our study was to assess the compositional variation of different types of abundantly available dairy side streams and their potential for direct utilisation as DF medium during milk filtration. Because of differences in their chemical compositions, we hypothesise that these media are likely to affect deposit structure and filtration performance in different ways. We further postulate that some of these media could qualify as an alternative DF medium compared with purified water.

At first, we carried out a screening of samples of dairy aqueous side streams to assess their range of compositional variation in industrial practice. After composition-based subcategorisation, the analytical data were compared to literature reports to identify processing side streams for which effects on filtration performance for MF/UF of milk cannot be predicted. Then, a milk protein fractionation trial with differently composed simulated DF media aimed at testing their effects on filtration performance, with flux and residual whey protein content as evaluation criteria. As media could be applied for intermediate washing cycles before a final water DF, we also investigated the reversibility of related changes for media that we assumed to affect flux, which is also inversely related to deposit resistance against permeation. This should further shed light on physicochemical mechanisms involved in the structuration of the deposit layer formed during milk filtration, which mainly consists of casein micelles and changes in relation to different milieu conditions (Vetier *et al*. 1988).

METHODS

Sampling of dairy side streams

In order to screen and characterise industrial dairy side streams, 89 samples of the above-mentioned media types were obtained from 38 different processing operations at 10 dairies located in Germany and Denmark. The conditions of processing were communicated as far as accessible and used for the evaluation in conjunction with the analytical results. Sampling was carried out by dairy processor personnel at up to three different points of time within a whole duration of six months. Samples were immediately cooled down and transported within 3 days refrigerated to our laboratory for prompt analysis.

Analysis of industrial side stream samples

The dry matter of the process media was determined gravimetrically using a CEM Smart Turbo microwave dryer (CEM GmbH, Kamp-Lintfort, Germany). Device settings were adjusted to 100% of the provided power, a total drying time of up to 10 min and a maximum of 105°C. The pH and conductivity value of the samples were determined at 10°C and 50°C as most relevant temperatures for filtration processes on a measuring device (Seven Multi, Mettler Toledo, Schwerzenbach, Switzerland) using a glass electrode (Inlab Expert Pt1000; Mettler Toledo) and a conductivity probe (Inlab 731; Mettler-Toledo). These methods are also suitable for the rapid/inline assessment of composition in dairy practice. The freezing point of the samples was determined using a thermistor cryoscope (CryoStar I; Funke-Gerber Labortechnik GmbH, Berlin, Germany) in a procedure according to DIN EN ISO 5764:2009-10. Conductivity and freezing point, as sum parameters for dissolved, dissociated substances and the number of particles, respectively, provide an estimate of the ionic strength of the fluids. For a detailed characterisation of the media concerning the ionic environment, the content of individual ion types was determined. For this purpose, relevant anions and related chemical groups (chloride, phosphate, citrate and sulphate) and cations (sodium, potassium, calcium and magnesium) were detected by ion chromatography according to Dumpler *et al*. (2017) with a limit of quantification of 3 mg/L for anions and 7.5 mg/L for cations. The content of lactose and lactate content was determined by an RP-HPLC method according to Dumpler *et al*. (2016) with limits of quantification of 0.03 g/L, respectively, 0.04 g/L. For this analysis and ion chromatography, ultrafiltration permeate samples were diluted 1:20 with ultrapure water (Millipore Milli-Q Integral 3; Merck KGaA, Darmstadt, Germany) to be within the calibration range of the method. Sample pH was adjusted to 2.00 (60% $HClO_4$) to precipitate proteins. The content of the major whey proteins of UF permeates and thermo-curd whey was determined by HPLC as described by Heidebrecht *et al*. (2018). Limits of quantification were 0.02 g/L for α-lactalbumin (α-La), 0.03 g/L for β-lactoglobulin B (β-Lg B) and 0.03 g/L for β-lactoglobulin A (β-Lg A). Sample pH was adjusted to 4.60 (0.1 M HCl) to precipitate potentially included caseins. All chromatographic analyses were performed after application of a syringe filter (RC-45/25 Chromafil® Xtra ϕ = 0.45 µm; Macherey-Nagel, Düren, Germany) on Agilent 1100 series chromatographs (Agilent Technologies, Waldbronn, Germany). The reduced pH prevented the prepared samples from microbial growth and related compositional changes during a maximum of 5 days of intermediate storage at 4°C.

DF media for application in filtration experiments

Pasteurised (72°C, 30 s) bovine skim milk from a local dairy plant (Molkerei Weihenstephan GmbH & Co. KG, Freising, Germany) was kept at 4°C until further use as starting retentate material. Firstly, we applied a simulated NF permeate ($NF_{high,pH\ 7}$) that was reconstructed from chemical salts and deionised water (Table 1). The content of the main monovalent ions was adjusted to the level reported for simulated milk ultrafiltrate by Dumpler *et al*. (2017). The content of multivalent ions was adjusted at a reduced level, to generally investigate this kind of shift of the ratio of ions, which is well-known for NF permeates. For comparison of calcium effects in relation to other components on filtration, we adjusted the calcium level that was found in hard tap water applied during comparable DF by Reitmaier *et al*. (2021). The chosen values also represent a good approximation to the maximum amounts detected in the carried out screening of industrial NF permeate samples. A 1:1 dilution of $NF_{high,pH\ 7}$ with deionised water was carried out to enable the investigation of an NF permeate with reduced ion content ($NF_{low,pH\ 7}$). We added small amounts of L-(+)-lactate solution, 40% in H_2O, for the adjustment of pH 7 for both of these simulated NF permeates. A pH of 5 was additionally adjusted for DF with $NF_{low,pH\ 5}$, as reduced pH values were observed in some of the industrial samples, which should negatively impact filtration performance. All chemicals used for reconstruction of media were of analytical grade and purchased from Sigma-Aldrich, Friedberg, Germany, except from KCl (Carl Roth, Karlsruhe, Germany).

Application of selected aqueous phases as DF media in skim milk MF

During cross-flow MF of 25 L skim milk at pilot scale, the ionic milieu was specifically changed by means of a 'constant-volume batch' DF with simulated NF permeates. This means that a continuously adapted supply with the corresponding DF medium maintained a constant retentate fill level during filtration (Lipnizki *et al*. 2002). In this application mode, each DF step represents the accomplishment of one volume turnover by the DF medium relative to the starting feed volume. Trials were conducted with a filtration rig described in detail by Kühnl *et al*. (2010) and Reitmaier *et al*. (2021). In brief, filtrations were carried out with a ceramic 23-channel membrane (ISOFLUX®,

Table 1 Formulation for the reconstruction of nanofiltration (NF) permeate with relatively high ion content ($NF_{high,pH\ 7}$) by chemical salts.

Salt		c (mM/L)								
c (g/L)	Formula	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	PO_4^{3-}	Lactate	Citrate	SO_4^{2-}
0.5	$K_3PO_4 \times H_2O$		2.17				2.17			
0.34	$K_3C_6H_5O_7 \times H_2O$		1.05						1.05	
1.8	KCl		24.14			24.14				
0.887	NaCl	15.18				15.18				
0.013	$MgSO_4 \times 7\ H_2O$				0.05					0.05
0.213	$MgCl_2 \times 6\ H_2O$				1.05	1.05				
0.653	$CaC_6H_{10}O_6 \times 5\ H_2O$			2.05				2.16		
	Total	15.18	27.36	2.05	1.10	40.37	2.17	2.16	1.05	0.05

0.14 mm, $A = 0.35$ m²; TAMI Industries, Nyons, France) at constant pressure conditions ($\Delta p_{TM} = 1.10^5$ Pa; $\tau_w = 150$ Pa). Before the adjustment of the filtration temperature with water by means of a water bath, respectively, by an ice-water-supplied heat exchanger, cleaning and preconditioning with alkaline performed as described by Kühnl *et al*. (2010). Diafiltration tests without a preconcentration step were conducted at 50°C for simulated NF permeates for which a change of the medium along the process to deionised water was applied to test the reversibility of induced effects by means of another milieu change. Nanofiltration permeates or deionised water were each stirred and gently preheated in a water bath (53 ± 1°C) for 15 min. To assess the filtration speed and the separation efficiency, the filtrate mass flow J was directly measured and the residual content of whey proteins in the retentate was determined by RP-HPLC (Dumpler *et al*. 2016). The specific mass-based flux (J) was calculated by Eqn (1) with the membrane surface area (A), measured permeate mass (dm) per time interval (dt).

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

Data analysis

Agilent ChemStation software (Rev. B.04.03) was used for data analysis of RP-HPLC chromatograms. Data were plotted with Sigmaplot 13 (Systat Software Inc., San Jose, CA, USA). To identify the compositional fluctuation of different industrial side stream media, the data presentation includes the minimum and maximum values observed in our analytical screening. Besides this span of the detected values, the mean and standard deviations of samples from different side stream groups are shown.

RESULTS AND DISCUSSION

Analytical characterisation of dairy side streams and evaluation of applicability for DF

Figure 1 (a) depicts pH values and conductivity of 89 industrial samples from 38 different processes categorised by the type of unit operation they were obtained from. Data additionally obtained from pasteurised skim milk are shown for comparison, as this can be referred to as a typical retentate milieu before applying DF. Figure 1 (b) depicts the relative contents of sodium and calcium in comparison with milk serum (Gaucheron 2011) to highlight changes of individual ionic species. As can be seen, significant compositional deviations from the native milk milieu were detected. A marked variation in composition was determined within the permeate groups derived from UF and NF, whereas a nearly complete washout of ions for RO permeates or vapour condensates was observed. For UF permeates, samples were found to be close to the milk milieu or revealed an increased ion content which was partially accompanied by a reduction of pH. Acidic and mildly alkaline pH conditions were found to be partially accompanied by a shift of ionic composition in NF or UF permeates samples, which can be mainly attributed to the source material used.

Therefore, to further assess the compositional variation of side streams within these main product categories accounting only for the unit operation, we applied a further categorisation of the media types related to the different typical source materials (skim milk, sweet whey and acid whey). Results of detailed compositional investigations are presented and discussed in the following using this subcategorisation. We observed that for UF permeates the compositional variation was mainly related to the source material (Table 2).

Samples obtained from skim milk UF mainly varied in calcium and phosphate contents, dependent on the temperature (10 or 50°C) during UF, but generally met the range of compositional data reported for the serum phase of milk. In comparison with UF permeates obtained at ~10°C, reduced amounts of calcium, citrate and phosphate were observed for UF permeates from a UF process carried out at 50°C. These data of industrial samples confirm experimental results from UF experiments performed at different temperatures reported by Dumpler *et al*. (2017). For UF permeates obtained from sweet (chymosin) whey, the compositional data reflect a variation depending on the composition of the source material and the related cheesemaking process. For UF permeates obtained from sweet (chymosin) whey, the compositional data reflect a variation depending on the composition of the source material and the related cheesemaking process. Reduced pH values are going along with increased amounts of calcium, phosphate, lactate or citrate arising from differently performed pre-acidifications and additions of $CaCl_2$ during chymosin cheese production. Ultrafiltration permeate obtained from acid (or sour) whey showed a reduced pH of 4.30–4.42, linked to the high lactate content. The high contents of calcium and phosphate in comparison with milk serum are typical for acid whey (Crowley *et al*. 2017). They have their origin in the release from casein micelles during the acidic fermentation of milk. The obtained compositional values furthermore compare well with data of different unfiltered acid whey streams recently reported by Chandrapala *et al*. (2015) and Menchik *et al*. (2019). A reduction of pH and an increase of the calcium content, which both apply for this medium, have separately been shown to foster deposit permeation resistance in skim milk filtration due to an increase of protein–protein or protein–membrane interactions (Kessler *et al*. 1982; Vetier *et al*. 1988; Rabiller-Baudry *et al*. 2005). The combination of both milieu modifications and a successive change towards these milieu conditions, as it occurs during DF, could lead to deviations from reported filtration behaviour. Based on the obtained compositional data, we consider it likely that DF

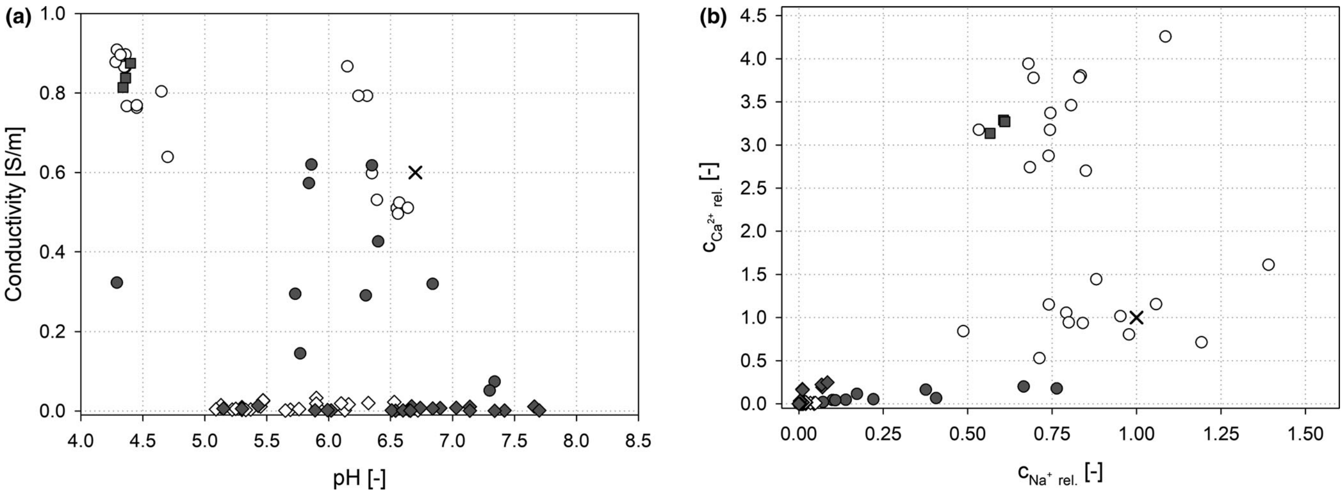

Figure 1 Conductivity and pH values (a) of single samples as well as their concentration of calcium and concentration of sodium ions in relation to milk serum (b). Values of a skim milk sample and a typical milk serum phase are additionally indicated as reference values (crosses). The sample classification according to the source process is indicated as follows: UF permeate (open circles); NF permeate (filled circles); RO permeate (open diamonds); vapour condensate (filled diamonds); and thermo-curd whey (open squares).

with UF permeates derived from acid whey will induce a significantly increased deposit resistance and related reduction of flux in comparison with deionised water or UF permeate obtained from skim milk or sweet whey. This renders the applicability of sour UF permeates as DF medium principally questionable. A reduction of pH before skim milk MF/UF or during DF can, however, also induce an altered product functionality of the obtained micellar casein concentrate due to the reduction of micellar calcium phosphate (Guinee *et al*. 2009; Schäfer *et al*. 2021). The controlled application of acid whey permeate as DF medium could offer the potential for a targeted modification of casein functionality in a similar manner, thereby replacing so far applied technical acidulants. The lactose content of UF permeates obtained from skim milk generally met values reported for milk. The partially observed reduction of the lactose content for whey permeates can be explained by variable cheesemaking methods regarding the extent of fermentation, curd washing and syneresis. A reduction of the lactose content during application of these media for DF can generally be expected to only slightly increase filtration performance due to a reduction of the viscosity of the serum phase (Adams *et al*. 2015). However, the detected lactose contents of at least 29.05 g/L confirm that a high protein purity of the obtained protein concentrate will not be achieved when applying UF permeate derived from milk or whey for DF during MF or UF.

Nanofiltration permeates significantly varied in composition between different processors and in the course of production time for samples derived from the same source (Table 3). Nanofiltration permeates from skim milk and sweet whey showed a high variation in pH from 5.7 up to 7.3, probably based on differences in the permeating components, mainly monovalent ions. Nanofiltration permeates obtained from sour whey had a lower pH of 4.3–5.7. This can be attributed to lactate, which also partially permeates. In comparison with the milk milieu, a relatively low conductivity and content of multivalent ion species and lactose were detected for these media types. Although data varied significantly, analytical values were generally found at a level that can generally be expected from typical NF process conditions. This means a membrane-dependent, but generally high rejection of all components of the milk, except monovalent ions. However, the data also demonstrate that dairy NF permeates considerably vary in practice and a kind of typical ionic composition was not found.

All in all, the analytical profiles varied depending on the membrane retention behaviour and type of source material and changed over industrial production time. It can therefore be expected that NF permeates used as DF medium varying in composition over time will change DF performance along production time. The low ionic strength and calcium content should result in a less intense deposit formation, whereas the partially observed reduced pH could induce an increase in deposit resistance. Several NF permeate samples contained a high concentration of calcium that was not counterbalanced by complex anionic species as in the natural milk milieu. This alteration of the ionic composition is based on the selective rejection of multivalent anions by NF membranes due to charge effects (‘Donnan effect’) (Roy 2018). This shift might be linked to an increased calcium activity, which should strengthen deposit formation when applied for DF. We conclude that application of NF permeates in DF could lead to a filtration performance established between

Table 2 Analytical results obtained from ultrafiltration (UF) permeate samples.

Analytical value	UF permeate (skim milk) from 3 sources, $n = 6$			UF permeate (sweet whey) from 2 sources, $n = 6$			UF permeate (sour whey) from 5 sources, $n = 12$			Thermo-curd whey from 1 source, $n = 3$		
	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max
pH 10°C (–)	6.54 ± 0.25	6.29	6.81	6.51 ± 0.09	6.40	6.63	4.43 ± 0.18	4.22	4.76	4.34 ± 0.07	4.3	4.42
pH 50°C (–)	6.42 ± 0.19	6.24	6.64	6.33 ± 0.14	6.15	6.55	4.43 ± 0.14	4.28	4.70	4.37 ± 0.03	4.34	4.40
Conductivity 10°C (S/m)	0.450 ± 0.026	0.423	0.493	0.605 ± 0.129	0.445	0.747	0.744 ± 0.088	0.647	0.949	0.770 ± 0.041	0.730	0.811
Conductivity 50°C (S/m)	0.517 ± 0.021	0.496	0.556	0.682 ± 0.154	0.510	0.867	0.838 ± 0.095	0.639	1.004	0.842 ± 0.031	0.814	0.875
Freezing point depression (m°C)	4845 ± 258	4550	5219	6655 ± 1328	5280	8450	6765 ± 766	5617	8261	7149 ± 221	6974	7398
Dry matter (%)	5.55 ± 0.25	5.32	5.92	6.89 ± 1.88	4.70	9.22	5.73 ± 0.59	4.79	6.76	6.11 ± 0.14	5.95	6.19
Sodium (mg/L)	425 ± 49	352	483	466 ± 164	241	687	380 ± 66	263	536	293 ± 12	280	301
Potassium (mg/L)	1277 ± 373	740	1572	1505 ± 472	841	2293	1585 ± 226	1216	1973	1681 ± 63	1612	1735
Magnesium (mg/L)	102 ± 36	60	151	130 ± 20	102	165	157 ± 26	103	199	156 ± 3	153	158
Calcium (mg/L)	341 ± 108	191	521	393 ± 112	257	582	1235 ± 181	975	1537	1166 ± 30	1132	1187
Chloride (mg/L)	1091 ± 237	629	1313	1255 ± 491	740	1776	1292 ± 180	946	1549	1253 ± 24	1225	1272
Phosphate (mg/L)	981 ± 92	836	1048	1094 ± 515	606	1722	1977 ± 238	1575	2295	2034 ± 48	1980	2071
Citrate (mg/L)	1078 ± 143	806	1179	1795 ± 788	889	2764	1085 ± 751	n.d.	2203	8 ± 8	n.d.	15
Sulphate (mg/L)	85 ± 14	68	107	145 ± 64	71	235	126 ± 60	43	250	105 ± 26	80	133
Lactose (g/L)	47.56 ± 2.37	45.63	50.80	54.88 ± 13.47	36.64	70.03	36.59 ± 5.39	29.05	43.67	44.39 ± 5.15	41.27	50.34
Lactate (g/L)	0.02 ± 0.01	n.d.	0.05	0.33 ± 0.14	0.10	0.48	6.56 ± 2.10	2.76	8.51	8.46 ± 0.36	8.05	8.71
α - La (g/L)	0.00 ± 0.01	n.d.	0.02	0.03 ± 0.03	0.01	0.08	0.03 ± 0.03	n.d.	0.07	0.20 ± 0.03	0.18	0.23
β - Lg B (g/L)	0.00 ± 0.00	n.d.	0.01	0.03 ± 0.03	n.d.	0.08	0.01 ± 0.01	n.d.	0.05	0.03 ± 0.01	0.02	0.03
β - Lg A (g/L)	0.00 ± 0.00	n.d.	n.d.	0.01 ± 0.02	n.d.	0.04	0.01 ± 0.01	n.d.	0.04	0.06 ± 0.01	0.05	0.06

n.d., not detected in a quantifiable concentration. For calculations of mean and standard deviation, this was evaluated as 0.

Table 3 Analytical results obtained from nanofiltration (NF) permeate samples.

	NF permeate (skim milk) from 2 sources, n = 2			NF permeate (sweet whey) from 2 sources, n = 6			NF permeate (sour whey) from 2 sources n = 2		
Analytical value	Mean	Min	Max	Mean ± SD	Min	Max	Mean	Min	Max
$pH_{10°C}$ (−)	6.11	5.82	6.4	6.75 ± 0.56	5.8	7.37	5.07	4.30	5.84
$pH_{50°C}$ (−)	6.06	5.77	6.35	6.66 ± 0.63	5.73	7.36	5.08	4.29	5.86
$Conductivity_{10°C}$ (S/m)	0.356	0.126	0.586	0.214 ± 0.129	0.047	0.366	0.451	0.329	0.573
$Conductivity_{50°C}$ (S/m)	0.382	0.145	0.618	0.243 ± 0.148	0.052	0.426	0.472	0.323	0.620
Freezing point depression (m°C)	1392	593	2190	865 ± 362	485	1438	1737	1381	2094
Dry matter (%)	0.28	0.01	0.54	0.50 ± 0.31	0.17	0.95	0.18	0.09	0.27
Sodium (mg/L)	182	35	329	92 ± 51	49	186	289	201	377
Potassium (mg/L)	728	85	1372	246 ± 163	95	523	1036	731	1340
Magnesium (mg/L)	15	3	26	10 ± 7	3	24	18	12	23
Calcium (mg/L)	40	7	72	28 ± 18	15	59	44	24	64
Chloride (mg/L)	750	385	1116	354 ± 360	45	805	1109	959	1260
Phosphate (mg/L)	85	46	125	69 ± 20	39	96	91	53	129
Citrate (mg/L)	32	30	35	57 ± 39	2	106	8	n.d.	16
Sulphate (mg/L)	9	9	10	6 ± 4	n.d.	11	n.d.	n.d.	n.d.
Lactose (g/L)	1.55	0.90	2.19	3.08 ± 2.56	n.d.	6.57	0.10	0.08	0.12
Lactate (g/L)	n.d.	n.d.	n.d.	0.02 ± 0.03	n.d.	0.06	1.44	0.47	2.41

n.d., not detected in a quantifiable concentration. For calculations of mean and standard deviation, this was evaluated as 0.

that of purified water and UF permeate derived from skim milk. In case of reduced pH or higher ion contents, a lower zeta potential and therefore reduced repulsion of casein micelles, as well as increased calcium content, should especially reduce the porosity of the protein layer deposited at the membrane surface and reduce flux (Rabiller-Baudry *et al*. 2005; Reitmaier *et al*. 2021).

Except from one sampled RO process, all of the RO permeates (Table 4), and evaporation condensates (Table 5), contained only small residual amounts of ions, lactose and lactate, mostly close to or below the detection limits. This makes them generally comparable to deionised water regarding milieu factors of relevant impact on filtration and casein functionality. The presented data for RO permeates obtained from acid whey are comparable to values derived from sour whey NF permeates (Table 3). This means that the retention for this RO process was not within typical membrane specifications. The producing company acknowledged a defect of the membrane. However, this observation shows that deviations from the expected composition can occur over the life cycle of membranes.

Application of simulated NF permeates in pilot-scale DF

Compositional fluctuations of NF permeate over time at single processors (Table 3) make the practical application unlikely to fit a controlled and uniform DF processing at an industrial scale. We tested different simulated NF permeates within the compositional range of the industrial samples on their impact on flux and milk protein fractionation when applied as DF medium in pilot-scale MF. The DF medium was varied in consecutive order during filtration to assess the impact of different compositional attributes during a DF with varying medium compositions. This should reflect the consecutive use of different filtration media in a row, obtained during regular production at variable times. The applied media varied in ionic strength, calcium content and pH to vary charge and zeta potential and overall hydration of casein micelles. Thus, significant composition-related changes in the filtration performance were expected. Firstly, we applied a simulated NF permeate that contained as many monovalent ions as simulated milk ultrafiltrate but reduced amounts of di- and especially multivalent ions ($NF_{high,pH\ 7}$) to test the hypothesised negative impact of this typical compositional shift on flux. Subsequent washing with a similar medium with reduced ionic strength ($NF_{low,pH\ 7}$) allowed for an investigation of a possible reversible reduction of the deposit resistance, which would indicate that milieu changes carried out in series will each impact filtration performance, even in opposite ways. This medium was subsequently applied at a reduced pH of 5 ($NF_{low,pH\ 5}$) to simulate a shift to acidic conditions. This pH level had also been detected in several samples within the analytical screening and is known to reduce flux in milk filtration. The reversibility of this type of milieu alteration was again checked by a final washing with deionised water, which is well known to decrease deposit resistance and increase flux in milk filtration. The overall set-up was 3 DF steps with $NF_{high,pH\ 7}$ → 3 DF steps

Table 4 Analytical results obtained from reverse osmosis (RO) permeate samples.

	RO permeate (skim milk) from 5 sources n = 12			RO permeate (sweet whey) from 7 sources n = 15			RO permeate (sour whey, defective membrane) from 1 source, n = 3		
Analytical value	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max
pH 10°C (−)	5.91 ± 0.38	5.43	6.65	5.36 ± 0.31	4.98	6.01	4.32 ± 0.06	4.26	4.37
pH 50°C (−)	5.96 ± 0.43	5.33	6.72	5.47 ± 0.32	5.09	6.13	4.32 ± 0.06	4.27	4.39
Conductivity 10°C (S/m)	0.012 ± 0.009	0.001	0.023	0.008 ± 0.008	0.001	0.032	0.163 ± 0.032	0.126	0.186
Conductivity 50°C (S/m)	0.013 ± 0.010	0.001	0.027	0.010 ± 0.009	0.001	0.033	0.179 ± 0.032	0.142	0.198
Freezing point depression (m°C)	114 ± 69	36	220	108 ± 87	24	318	882 ± 158	700	976
Dry matter (%)	0.00 ± 0.00	0.00	0.00	0.05 ± 0.19	0.00	0.75	0.40 ± 0.11	0.3	0.5
Sodium (mg/L)	8 ± 7	2	24	6 ± 6	2	22	37 ± 5	33	42
Potassium (mg/L)	21 ± 20	n.d.	47	12 ± 12	n.d.	41	275 ± 57	231	339
Magnesium (mg/L)	1 ± 0	n.d.	2	1 ± 1	n.d.	1	10 ± 2	9	11
Calcium (mg/L)	3 ± 3	n.d.	7	n.d. ± 1	n.d.	3	80 ± 10	69	90
Chloride (mg/L)	21 ± 24	n.d.	55	22 ± 31	n.d.	120	267 ± 27	239	293
Phosphate (mg/L)	7 ± 6	n.d.	14	3 ± 4	n.d.	18	136 ± 2	133	137
Citrate (mg/L)	5 ± 5	n.d.	12	2 ± 4	n.d.	14	83 ± 65	10	136
Sulphate (mg/L)	1 ± 0	n.d.	2	n.d. ± 1	n.d.	2	3 ± 5	n.d.	8
Lactose (g/L)	0.23 ± 0.23	n.d.	0.66	0.06 ± 0.10	n.d.	0.37	2.69 ± 0.56	2.04	3.02
Lactate (g/L)	0.00 ± 0.00	n.d.	0.01	0.00 ± 0.00	n.d.	0.01	0.64 ± 0.24	0.44	0.90

n.d., not detected in a quantifiable concentration. For calculations of mean and standard deviation, this was evaluated as 0.

Table 5 Analytical results obtained from vapour condensate samples.

	Vapour condensate (skim milk) from 6 sources, n = 14			Vapour condensate (sweet whey) from 3 sources, n = 8		
Analytical value	Mean ± SD	Min	Max	Mean ± SD	Min	Max
pH 10°C (−)	6.77 ± 0.63	5.6	7.56	6.88 ± 0.98	5.23	7.64
pH 50°C (−)	6.62 ± 0.64	5.43	7.7	6.59 ± 0.89	5.15	7.66
Conductivity 10°C (S/m)	0.002 ± 0.002	0.001	0.007	0.007 ± 0.002	0.005	0.010
Conductivity 50°C (S/m)	0.003 ± 0.003	0.001	0.013	0.008 ± 0.003	0.006	0.012
Freezing point depression (m°C)	19 ± 24	5	95	43 ± 12	24	64
Dry matter (%)	0.00 ± 0.00	0.00	0.00	0.00 ± 0.00	0.00	0.00
Sodium (mg/L)	2 ± 1	1	5	2 ± 1	1	4
Potassium (mg/L)	1 ± 3	n.d.	11	1 ± 1	n.d.	2
Magnesium (mg/L)	1 ± 1	n.d.	4	1 ± 1	n.d.	1
Calcium (mg/L)	1 ± 4	n.d.	12	n.d. ± 0	n.d.	n.d.
Chloride (mg/L)	1 ± 2	n.d.	6	1 ± 2	n.d.	4
Phosphate (mg/L)	2 ± 4	n.d.	15	1 ± 1	n.d.	4
Citrate (mg/L)	1 ± 3	n.d.	11	n.d. ± 1	n.d.	2
Sulphate (mg/L)	0 ± 0	n.d.	1	1 ± 1	n.d.	3
Lactose (g/L)	0.04 ± 0.10	n.d.	0.38	0.02 ± 0.03	n.d.	0.08
Lactate (g/L)	0.00 ± 0.01	n.d.	0.02	0.01 ± 0.01	n.d.	0.02

n.d., not detected in a quantifiable concentration. For calculations of mean and standard deviation, this was evaluated as 0.

with $NF_{low,pH\ 7}$ → 3 DF steps with $NF_{low,pH\ 5}$ → 2 DF steps with deionised water. The consecutive variation of the DF medium allowed for an investigation of the reversibility of each of the induced effects that were postulated to be contrary for each of the milieu changes. This approach of media changes within this trial obviously leads to different starting conditions regarding deposit structure for each of the applied media but allows for the assessment of a certain set of variable DF media.

As hypothesised, $NF_{high,pH\ 7}$ and $NF_{low,pH\ 5}$ reduced the flux level, whereas $NF_{low,pH\ 7}$ and deionised water were found to increase the flux (Figure 2). The observed flux changes in comparison with the starting level (DF step 0) were −9% after DF step 4, +7% after DF step 7, −9% after DF step 9 and +2% after DF step 11 and seem to be relatively small. However, when considering the difference to the level achieved by the previous DF medium, a significant impact of media composition on the flux can be concluded. A shift of +19% by $NF_{low,pH\ 7}$, −16% for $NF_{low,pH\ 5}$ and +13% for deionised water in relation to the previously applied DF medium was achieved. Besides the milieu change, the continuous reduction of the whey protein content during the consecutive change of the DF medium (Figure 2) could also have affected the deposit structure and flux. However, we consider a relevant contribution unlikely as the reduction of major whey proteins was found to be on a comparable level to similar filtration trials with the application of one DF medium throughout (Reitmaier *et al*. 2021). This furthermore indicates a minor impact of this type of DF medium on whey protein transmission and applicability for milk protein fractionation by MF. However, the obtained results show that the variation of ionic strength/calcium content and pH in the ranges observed for NF permeates of different sources, in general, can markedly affect flux when applied for DF during skim milk MF.

DISCUSSION

The conducted analytical screening revealed that dairy side streams from different industrial processors, also when stemming from one single category of unit operation, can markedly vary in compositional details. Differences were found to depend on the type and condition of the applied processing plant, for example intactness of membranes. Only intact NF and RO membranes produce a permeate quality close to the expected composition, that is deionised water in the case of RO. This means that tight processing control of side streams' composition would be required, for example by measuring the conductivity of the membranes' permeate streams at various positions in larger membrane units to obtain early warning signs in case of membrane defects, for instance. Ultrafiltration permeate is a candidate for casein/whey protein fractionation without changing the native environmental conditions to maintain functional protein properties. However, the composition of UF permeates varied in dependence of the source material (sweet or sour whey), its pretreatment (e.g. addition of processing aids like citrates) and compositional uniformity over time (depending on the portfolio of cheeses produced by one manufacturer). Thus, the substitution of deionised water for DF by UF permeate to reduce water demand will only result in a virtually native composition of the casein product if obtained from unmodified skim milk. The varying flux level during the DF trial with simulated NF permeates demonstrates significant effects of slight variations of ionic composition on filtration performance, and thus, the relevance of the compositional variability of NF or UF permeates for their applicability as DF medium. The observed effects can be related to the permeates' ion contents and pH affecting colloidal interactions of casein micelles deposited on the membrane. Interestingly, the decrease of flux was, however, reversible upon a subsequent reduction of the ionic strength or calcium content, respectively, increase of pH. This also means that DF media applied one after the other in series can each affect deposit resistance, even in opposite directions, as long as the deposited material was not irreversibly cross-linked. To the best of our knowledge, this notable effect has not been reported in published works so far. Most industrially obtained RO permeates and vapour condensates can be considered as suitable DF medium allowing for direct substitution of deionised water without the generation of significant differences in process performance or product functionality.

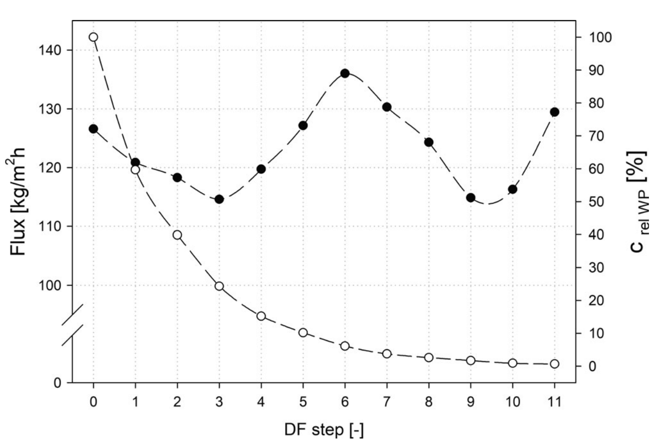

Figure 2 Course of flux (filled circles) and relative residual concentration of WP in the retentate (open circles) over progress of DF under variation of media (steps 1–3 with $NF_{high,pH\ 7}$; steps 4–6 with $NF_{low,pH\ 7}$; steps 7–9 with $NF_{low,pH\ 5}$; steps 10–11 with deionised water).

CONCLUSIONS

Analytical data obtained for dairy side streams revealed that compositional variability was wider than originally expected but generally acceptable for DF media. Ultrafiltration permeates should only be considered if stemming from a process without compositional manipulations during prior milk processing steps, for example by the addition of chymosin, acidulants or citrate. Otherwise, the casein fraction's functionality and process performance during milk protein fractionation by DF would be affected. Side streams containing traces of ionic species can be considered as applicable for DF yielding a reliably high filtration performance. If composition is stable and low in contents of ions and acidic components, NF or RO permeates and evaporation condensates could be applied for DF. This could help to significantly reduce the water footprint by replacing so far typically applied deionised water without requiring purification prior to the application as a DF medium. If stable DF processing performance is aimed at, tight processing control regarding DF media composition would be required. However, possible regional legislative limitations and the control of potential microbiological risks have to be considered for practical implementation.

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CONFLICT OF INTEREST

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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; Supervision; Validation; Writing – review & editing.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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