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Minor acidification of diafiltration water using various acidification agents affects the composition and rennet coagulation properties of the resulting microfiltration casein concentrate

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ABSTRACT

Cheese made from microfiltration (MF) retentate may suffer from textural defects due to a high Ca concentration. The reduction of colloidal minerals by the acidification of milk before MF at pH below 6.0 has been well documented in the literature. This process, however, creates less valuable side streams to the MF process and induces changes in the casein micelles that negatively affect their coagulation properties. The objective of this study was to determine whether a minor reduction in pH by using different acidifiers in the diafiltration (DF) water could induce changes in composition and renneting properties of the MF retentate. A 2-stage filtration process was used, with the first designed to increase the casein concentration to 8% and the second to slightly reduce the casein concentrate by 0.1 pH unit by DF, without influencing the total protein concentration. Four acidifying agents were tested during DF: lactic acid, hydrochloric acid, citric acid, and carbon dioxide. Diafiltration with water was used as a reference. At the start of DF, the retentates of acid DF had a slightly reduced pH, with an average of 0.09, whereas the pH of the reference retentate increased by an average of 0.07 unit. The reference retentate regained its starting pH by the end of DF. The carbonated retentate gradually increased in pH during processing, whereas the pH of the lactic, hydrochloric, and citric acid retentates remained constant. The permeate from the lactic acid and carbonated treatments had a reduced whey protein content compared with the reference. The total P and inorganic phosphate were lowered in the retentate by using carbonation. The total amount of Mg and Na were lowered in the retentate by using citric acid. The ionic Ca content in the retentate increased with use of lactic or hydrochloric acid. The type of acidifier used reduced the rennet clotting time. Combined acidified diafiltration with a slight reduction affects the permeate composition and improves the retentate clotting time despite the minimal mineral modification.
Key words: casein concentrate, diafiltration, acidifying agent, composition, coagulation

INTRODUCTION

Microfiltration (**MF**) is of increasing interest for the cheese industry, mostly for its protein separation selectivity, where high casein retentates and native whey protein (**WP**) permeates can be obtained (Lagrange et al., 2015). Production of cheese from MF retentate is more profitable in terms of cost reduction compared with conventional cheesemaking (Papadatos et al., 2003).

The MF process influences the chemical composition of the obtained retentate; as the casein concentration increases, the colloidal and casein-bound mineral contents and buffer capacity also increase (Salaün et al., 2005; Marella et al., 2013). Cheese made from MF retentate shows texture and flavor defects due to the high mineral content (Neocleous et al., 2002; Heino et al., 2010; Schreier et al., 2010; Soodam and Guinee, 2018). Therefore, adjustment of the mineral content of the concentrated cheese milk before cheesemaking is necessary. The reduction of mineral content in MF retentate could be achieved by acidification of the milk (Law and Leaver, 1998), acidified diafiltration (**DF**; Holt et al., 1986; Hurt and Barbano, 2010; Alexander et al., 2011; Ferrer et al., 2014; Boiani et al., 2017), or a combination of the two (St-Gelais et al., 1992). The effect of acidifying milk to pH 6.0 and below, where changes in mineral balance are apparent, is well documented in the literature. The solubilization of colloidal Ca phosphate (**CCP**) from the casein micelle upon acidification of milk causes an increase in the content of soluble minerals such as various forms of pH-dependent

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phosphates and Ca^{2+} (Le Graët and Gaucheron, 1999; Dalgleish and Corredig, 2012). The rate of solubilization increases at a pH of approximately 5.6 to 5.8 (De la Fuente, 1998; Li and Corredig, 2014). Colloidal phosphate is fully solubilized at pH 5.2, whereas Ca requires a further decrease in pH for complete solubilization. As casein concentration increases, an even further reduction in pH is required to induce solubilization of CCP (Le Graët and Gaucheron, 1999). The type of acidifier used plays a role in the ionic composition of the serum phase, which further affects the functionality of the casein micelle (Broyard and Gaucheron, 2015). Guillaume et al. (2004) and De la Fuente (1998) reported that the Ca^{2+} concentration in the serum phase of the milk was a consequence of the acidifier type used.

Membrane filtration of highly acidified milk increases the risk of fouling, which affects the performance by reducing the flow rate (Ng et al., 2017). Moreover, filtration of highly acidified milk yields a relatively acidic permeate, the value of which for further applications is reduced. Thus, this process is not a practical solution for industries when the side streams of the MF process are intended for usage in other applications. The DF process, which aims to remove lactose and soluble minerals and increase protein content, alters the mineral balance of the casein micelle. Ferrer et al. (2014) showed that the extent of DF (0.5 and 1 diavolume; **DV**) decreased the soluble Ca and P contents in the retentate. These authors also reported a disruption of the casein micelle and increased solubility of κ-, β-, and α_S-CN, a reduced rennet gel stiffness, and an increased gelation time. Boiani et al. (2017) reported that by altering the DF medium (i.e., by adding citrate to the DF water), the total P:Ca ratio increased, and this shifted the casein phosphate nanoclusters signals recorded by 31P nuclear magnetic resonance compared with using regular DF water. They also observed a shift toward an acidic pH with a reduction in Ca and P contents when using citric acid as the DF medium. Therefore, addition of modifying agents to the DF water and the DF factor could be used to modify the composition of casein concentrates.

The aim of the present study was to find an intermediate solution with capabilities to both (1) allow a slight reduction in milk pH as a prepreparation step for further cheesemaking to improve the texture of cheese made from MF milk without disruption of the casein micelle and (2) maintain a valorized stream of the MF process (i.e., resulting in a nonacidic permeate). The objective of this study was thus to investigate an MF-DF process, where different acidifiers added to the DF water were tested for their potential effect on the resulting casein concentrate retentate and permeate. To the authors' knowledge, this is the first comprehensive study comparing the effect of different acidifying agents on the retentate and permeate composition following an acid DF process.

MATERIALS AND METHODS

Sample Preparation

Figure 1A illustrates the 2-stage production process of the casein concentrate used in this study. Fresh milk, obtained from the Animal Production Experimental Centre at the Norwegian University of Life Sciences (Ås), was skimmed (Westfalia Separator AG, MSD50-01-076, Oelde, Germany), pasteurized (A3-HRB, Alfa Laval, Lund, Sweden), and microfiltered (UF/MF pilot MCC RV 01118340, APV, Slikeborg, Denmark) at 50°C using a 0.14-μm ceramic membrane (Inside Céram, Tami Industries, GEA, Nyons, France) at uniform transmembrane pressure (51 ± 4 kPa) to 8 ± 0.1% (wt/wt) casein concentrates, as previously described by Gaber et al. (2020). The macrocomposition of the retentate during MF was determined by a MilkoScan FT1 (CombiFoss 6500, Foss, Hillerød, Denmark) using Fourier transform infrared analysis as a fast method to monitor the protein concentration. The casein concentrates were distributed in 1-L sterile containers and kept at 4°C before further filtration.

The DF process was initiated after MF concentration by adding 0.3 DV of DF medium to the MF retentate. The DV was chosen based on a target lactose content of the MF-DF retentate of 3.9 ± 0.1%. Tap water was used as the reference DF medium to mimic industrial practice. Acidified DF medium was tap water with the addition of agents as described below. Filtration was then continued at 50°C using a Labscale TFF system (Millipore, Oslo, Norway), with a Pellicon XL Cassette and a Biomax membrane (500 kDa, cat. no. PBX500C50; Millipore) at an inlet and outlet pump pressure of ~3 bar. The recirculation of the casein concentrates in the feed tank and collection of permeate were initiated after discarding the first 30 mL of retentate and permeate from the system. The concentration continued until the volume of the collected permeate corresponded to the volume of added acidified water (measured using a graduated cylinder). The membranes were cleaned according to the manufacturer's instructions.

The individual acidifying agents in tap water used for DF were (1) 10 m*M* lactic acid (DL-lactic acid, 85% wt/wt, Sigma-Aldrich, St. Louis, MO), (2) 10 m*M* HCl (hydrochloric acid, ACS reagent, 37%, Sigma-Aldrich), (3) 10 m*M* citric acid (10% wt/wt citric acid monohydrate solution, citric acid monohydrate ACS reagent, ≥99.0%, Sigma-Aldrich), and (4) 1.69 g/L CO_2. The carbonation of water with CO_2 was performed using a

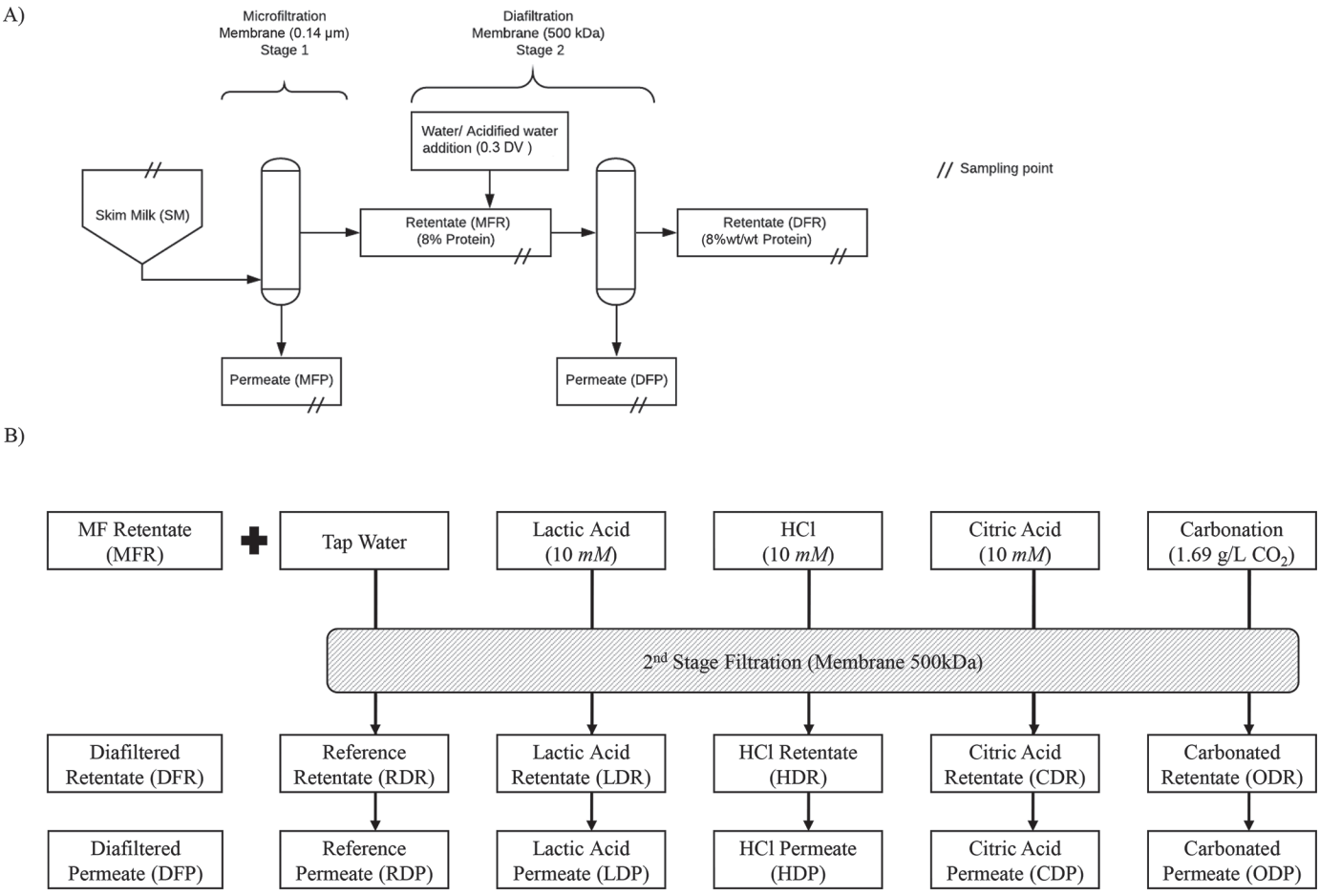

Figure 1. Schematic representation of (A) 2-stage filtration process of microfiltration (MF) and diafiltration (DF) and (B) details of DF treatments and nomination. DV = diavolume.

SodaStream (Aqvia, AGA, Stockholm, Sweden). Adjustment for the concentration of CO_2 was measured using an PBA-B instrument connected to a CarboQC ME module with a PFD filling device and operated through Generation M instrument software version 2.42 (Anton Paar, Graz, Austria). The concentration of CO_2 used was based on a decrease in the pH of the DF water to 4.6. The selected concentration of each acidifying agent was previously adjusted to correspond to the amount required to obtain a decrease in pH of the casein concentrate by 0.1 unit after the addition of acidified water. Continuous pH measurements were performed during acid DF using a 742020 Hach SensIon+ PH31 meter with 5011T probe (Lange GmbH, Dusseldorf, Germany) connected to LabCom V2.1 software (Lange GmbH).

The retentates and permeates obtained from the MF and DF were abbreviated as follows (Figure 1B): (1) MF retentate (MFR) and permeate (MFP), (2) DF reference retentate (**RDR**) and permeate (**RDP**), (3) DF lactic acidified retentate (**LDR**) and permeate (**LDP**), (4) DF HCl retentate (**HDR**) and permeate (**HDP**), (5) DF citric acid retentate (**CDR**) and permeate (**CDP**), and (6) DF carbonated retentate (**ODR**) and permeate (**ODP**). The skim milk (Swearingen et al., 2004), retentate, and permeate from the various MF and DF were analyzed for their different compositions.

Compositional Analysis

Total N, NPN, and noncasein N contents of all samples were determined using the Kjeldahl method (IDF, 2001, 2004, 2014). True protein (Guinee et al., 2002) was calculated by subtracting NPN from total N. Casein was calculated by subtracting noncasein N from total N. Undenatured WP was calculated by subtracting NPN from noncasein N. The results were multiplied by the factor 6.38 to calculate the percentage of protein. The identification and quantification of the protein composition were performed using capillary electrophoresis in

combination with the Kjeldahl results as described by Jørgensen et al. (2016). The moisture content of the skim milk, retentate, and permeate from MF and DF was determined according to IDF (2010) method 26A, and TS were calculated accordingly. Organic acids and carbohydrates were quantified by HPLC as described by Moe et al. (2013).

Mineral Analysis

The total contents of Ca, P, K, Na, and Mg were quantified by inductively coupled plasma MS as described by Jørgensen et al. (2015). Inorganic phosphate ($\mathbf{P_i}$) was analyzed using Agilent (G1600AX) capillary electrophoresis with ChemStation software (Agilent Technologies, Waldbronn, Germany) according to Izco et al. (2003) and with modifications as described by Gaber et al. (2020). Calcium ion activity was determined using an Orion 97-20 calcium ion selective electrode (Calcium Ionplus Sure-Flow Plastic Membrane Combination ISE, Thermo Scientific, Chelmsford, MA) with an mV meter (PHM290, pH-STAT Controller, MeterLab, Radiometer Analytica, Copenhagen, Denmark).

Rennet Coagulation Properties

The rennet coagulation properties of the skim milk and retentates from MF and DF were measured using a Formagraph (Lattodinamografo; Foss Italia SpA, Padova, Italy) as described by Inglingstad et al. (2014). A 10-mL aliquot of a casein concentrate sample was incubated at 32°C for 30 min in the sample cuvette, followed by addition of 200 μL of rennet (CHY-MAX; Chr. Hansen A/S, Hørsholm, Denmark) previously diluted 1:50 in acetate buffer (pH 5.6), and was immediately analyzed for 45 min at 32°C. Coagulation analysis included the rennet clotting time (**RCT**; min), curd firming time shown as time from RCT until the width of the bifurcate was 20 mm, and curd strength (in mm of distance) of the bifurcate after 30 min of coagulation.

Statistical Analysis

The entire experiment was carried out in 3 filtration replicate blocks with 3 separate milk batches. Samples obtained from MF and DF of each batch were analyzed in triplicate. The results are the averages of those obtained from the 3 replicate blocks. Statistical significances for the DF treatments were evaluated using ANOVA at $P < 0.05$ with the DF treatment as a fixed factor and the replicate block as a random factor. The mean values were compared using a Tukey pairwise comparison test, and all statistical data were processed using packages and functions in R Studio (version 1.1.456, https://rstudio.com/products/rstudio/download/).

RESULTS

Change in Retentate pH During DF

Throughout the DF process, the pH value differed significantly ($P < 0.05$) between the reference and the different acidification treatments (Figure 2). By adding acidified DF water to the MF retentate, the pH of the retentate instantly decreased by ~0.1 unit, whereas the reference sample increased by ~0.1 pH unit after water addition (Table 1). Throughout the DF process and removal of permeate, the pH of the reference retentate decreased to regain its starting value of pH 6.6 ± 0.03. In contrast, the pH of HDR and CDR remained relatively stable at 6.47 ± 0.02 and 6.43 ± 0.06, respectively. The LDR showed a slight reduction in pH during DF to 6.43 ± 0.1, and the ODR pH increased throughout the DF process to 6.5 ± 0.01. The LDR and CDR samples had a significantly ($P < 0.05$) lower end pH compared with the reference sample.

Protein Composition of the Retentate and Permeate

The protein concentration returned to 8% (wt/wt) after DF (Table 2). The contents of true protein, casein, and protein fractions (Supplemental Table S1, https://doi.org/10.3168/jds.2020-18237) in the retentates were not influenced by the DF medium. However, the total WP content in the retentates was significantly ($P < 0.05$) reduced in the HDR and ODR compared with the RDR.

The protein compositional analysis of the permeate (Table 2) showed no casein leakage after MF and DF, but the different acid DF influenced the amount of true protein (total WP) in the permeate. The specific contents of α-LA and β-LG in the permeate (Table 3) were significantly ($P < 0.05$) reduced in the LDP and ODP samples compared with the RDP.

Mineral Solubilization During Acid DF

The mineral composition of the retentates and the permeates from the 2-stage filtration process is shown in Table 4 and Figure 3, respectively. The relatively large milk batch variation in mineral composition caused large standard deviations within treatments. The total contents of Ca and K in the retentate were not affected by the method of DF compared with the reference. The total contents of Mg, Na, and P in the retentate were

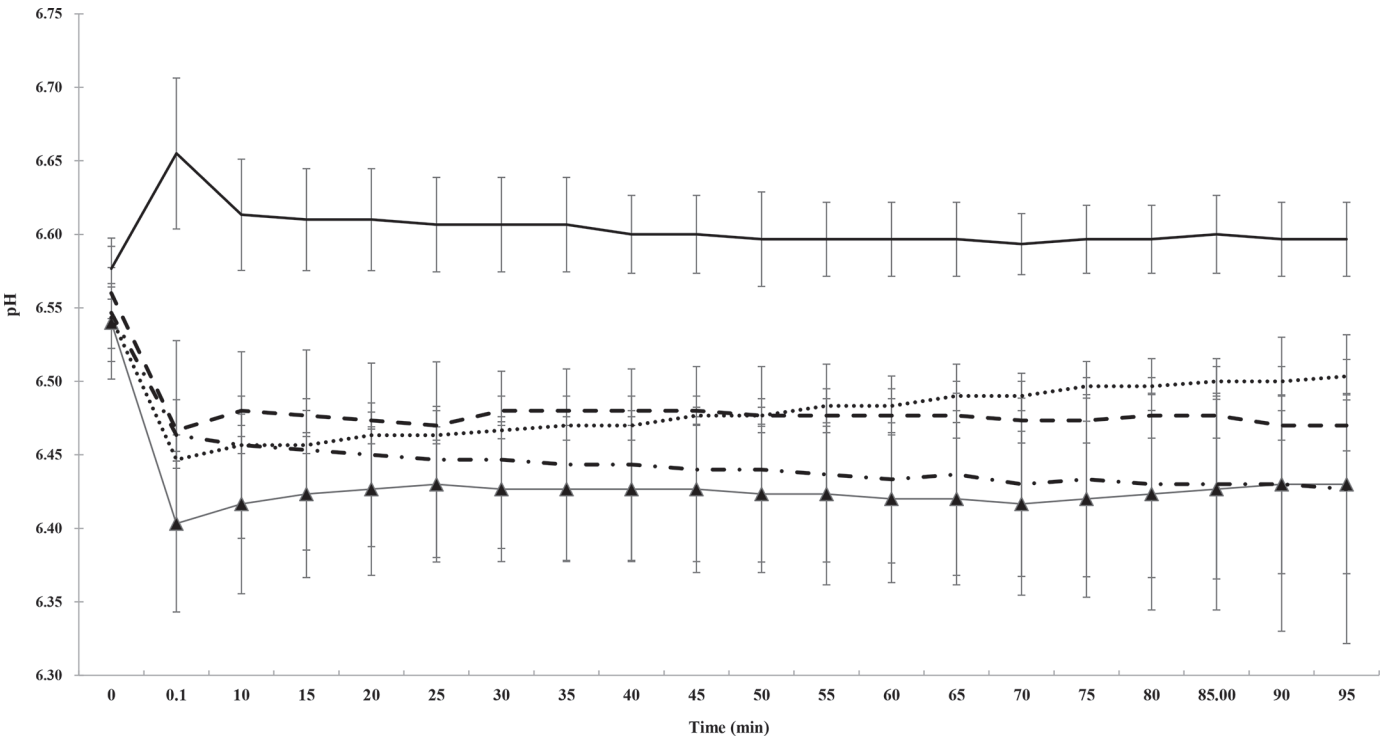

Figure 2. Change in pH during the diafiltration process of microfiltration retentate. — reference; —·— lactic acid; — — HCl; —▲— citric acid; ···· carbonated water. Means ± SD.

influenced by the type of acid DF, and DF with carbonated water had the greatest effect compared with other treatments. Acidifying the DF water with CO_2 reduced the total Mg and Na contents of the ODR, which were significantly ($P < 0.05$) lower than in CDR, which had the highest Mg and Na contents in the DF retentates. The CDR also had a significantly ($P < 0.05$) higher total Na content compared with LDR. The P and P_i contents were influenced by the DF medium, and ODR had significantly ($P < 0.05$) reduced total P and P_i contents compared with RDR. Diafiltration influenced the Ca^{2+} content of the DF retentate; LDR and HDR had a significantly ($P < 0.05$) higher Ca^{2+} concentration than RDR, which had the lowest Ca^{2+} content. The ODR did not significantly ($P < 0.05$) differ in Ca^{2+} concentration from any of the other retentates.

Table 1. Effect of acidifying agents used during diafiltration (DF) of casein concentrates on 0.1-pH changes at instant (Change) and at the end point (End) of the DF process and on rennet coagulation properties (RCP) as measured by Formagraph[1,2]

			Retentate				
Item	SM	MFR	RDR	LDR	HDR	CDR	ODR
pH							
Start	6.56 ± 0.08	6.55 ± 0.05	6.57 ± 0.02	6.54 ± 0.04	6.55 ± 0.01	6.51 ± 0.02	6.54 ± 0.02
Change[3]			6.66 ± 0.01^{a}	6.46 ± 0.06^{b}	6.46 ± 0.02^{b}	6.40 ± 0.06^{b}	6.44 ± 0.00^{b}
End			6.59 ± 0.02^{a}	6.42 ± 0.1^{b}	6.47 ± 0.01^{ab}	6.43 ± 0.06^{b}	6.5 ± 0.01^{ab}
RCP[4]							
RCT (min)	25.1 ± 2.4	14.2 ± 0.4	14.5 ± 0.5^{a}	10.4 ± 2^{c}	11.5 ± 1.4^{bc}	11.6 ± 0.4^{bc}	12.2 ± 1.9^{b}
A_{30} (mm)	5.84 ± 3.9	48.1 ± 2.9	47.2 ± 2.5	47.5 ± 2.4	49.2 ± 2.8	45.5 ± 3.9	47.1 ± 2.7
K_{20} (min)	15.86 ± 0.5	2.2 ± 0.3	2.1 ± 0.1	1.8 ± 0.3	1.9 ± 0.2	2 ± 0.3	2.2 ± 0.5

[a–c]Means within a row with different superscripts differ according to Tukey's pairwise comparison ($P < 0.05$).

[1]Lattodinamografo (Foss Italia SpA, Padova, Italy).

[2]SM = skim milk; MFR = microfiltered retentate. DF treatments of the retentate were as follows: RDR = reference; LDR = lactic acid retentate; HDR = HCl retentate; CDR = citric acid retentate; ODR = carbonated retentate.

[3]Instant change in pH due to addition of DF medium.

[4]RCT = rennet clotting time; A_{30} = firmness of the gel; K_{20} = curd firming time.

Table 2. Effect of different acidifying agents used for diafiltration (DF) on the composition of the retentate and the permeate compared with skim milk (SM) and microfiltered retentate (MFR) and permeate (MFP)

			Retentate[1]				
Item	SM	MFR	RDR	LDR	HDR	CDR	ODR
Composition (%)							
CP	3.44 ± 0.32	8.39 ± 0.11	8.51 ± 0.30	8.71 ± 0.66	8.65 ± 0.83	8.65 ± 0.28	8.52 ± 0.67
True protein	3.24 ± 0.32	8.22 ± 0.11	8.38 ± 0.19	8.54 ± 0.66	8.49 ± 0.83	8.48 ± 0.27	8.34 ± 0.67
CN	2.7 ± 0.16	7.53 ± 0.19	7.54 ± 0.17	7.75 ± 0.59	7.72 ± 0.77	7.69 ± 0.28	7.58 ± 0.64
Whey protein (WP)	0.53 ± 0.07	0.68 ± 0.14	0.83 ± 0.03^{a}	0.78 ± 0.08^{ab}	0.76 ± 0.06^{b}	0.79 ± 0.02^{ab}	0.76 ± 0.04^{b}
WP:CN	0.20	0.09	0.11	0.10	0.09	0.10	0.10
TS	9.06 ± 0.72	14.03 ± 0.18	13.42 ± 0.8	13.49 ± 0.78	13.25 ± 0.9	13.34 ± 0.3	13.01 ± 0.66
Moisture	90.93 ± 0.72	85.96 ± 0.18	86.57 ± 0.80	86.50 ± 0.78	86.74 ± 0.9	86.65 ± 0.3	86.98 ± 0.23
Lactose	5.15 ± 0.29	4.15 ± 0.7	3.39 ± 0.16	3.57 ± 0.02	3.57 ± 0.05	3.61 ± 0.15	3.6 ± 0.13
Organic acid (mg/L)							
Lactic acid	ND[2]	ND	ND	313.87	ND	ND	ND
Citric acid	2,179.40	1,949.61	1,627.07^{b}	1,796.14^{b}	1,763.56^{b}	2,041.22^{a}	1,754.71^{b}
			Permeate[3]				
		MFP	RDP	LDP	HDP	CDP	ODP
Composition (%)							
CP		0.68 ± 0.03	0.19 ± 0.01	0.16 ± 0.03	0.19 ± 0.03	0.19 ± 0.02	0.17 ± 0.01
True protein		0.49 ± 0.02	0.07 ± 0.05^{a}	0.03 ± 0.00^{b}	0.04 ± 0.01^{ab}	0.03 ± 0.01^{ab}	0.02 ± 0.01^{b}
Moisture		93.91 ± 0.30	95.74 ± 0.14	95.66 ± 0.07	95.66 ± 0.18	95.64 ± 0.04	95.85 ± 0.23
TS		6.08 ± 0.30	4.25 ± 0.14	4.33 ± 0.07	4.33 ± 0.18	4.35 ± 0.04	4.14 ± 0.23
Lactose		5.06 ± 0.19	4.01 ± 0.08	3.79 ± 1.57	3.87 ± 0.14	4.07 ± 2.34	3.85 ± 1.04
Organic acid (mg/L)							
Lactic acid		ND	ND	306.76	ND	ND	ND
Citric acid		2,012.49	1,491.93^{b}	1,400.30^{b}	1,430.96^{b}	1,817.89^{a}	1,415.85^{b}

^{a,b}Means within a row with different superscripts differ according to Tukey's pairwise comparison ($P < 0.05$).

[1]DF treatments of the retentate: RDR = reference; LDR = lactic acid retentate; HDR = HCl retentate; CDR = citric acid retentate; ODR = carbonated retentate.

[2]Not detected.

[3]DF treatments of the permeate: RDP = reference; LDP = lactic acid permeate; HDP = HCl permeate; CDP = citric acid permeate; ODP = carbonated permeate.

Table 3. Calculations of protein compositions in skim milk, microfiltration (MF) retentate, and diafiltered (DF) permeate based on capillary electrophoresis results[1]

	MF permeate			DF permeate[2]																
				RDP			LDP			HDP			CDP			ODP				
Item	RC	Adj TPC	Adj MEC	RC	Adj TPC	Adj MEC	RC	Adj TPC	Adj MEC	RC	Adj TPC	Adj MEC	RC	Adj TPC	Adj MEC	RC	Adj TPC	Adj MEC	RC	Adj TPC	Adj MEC
α-LA	29.64^{c}	0.134	0.13	47.09^{b}	0.033^{a}	0.032^{a}	53.1^{ab}	0.015^{b}	0.017^{b}	51.96^{ab}	0.023^{ab}	0.024^{ab}	53.6^{ab}	0.021^{ab}	0.021^{ab}	56.86^{a}	0.016^{b}	0.018^{b}			
β-LG	70.35^{a}	0.315		52.9^{b}	0.037^{a}	0.038^{a}	46.89^{bc}	0.014^{b}	0.012^{b}	48.03^{bc}	0.021^{ab}	0.020^{ab}	46.39^{bc}	0.018^{b}	0.021^{ab}	43.13^{c}	0.011^{b}	0.011^{b}			
Sum	100			100						100			100			100				

[a–c]Means of protein concentration within the same row with different superscripts differ ($P < 0.05$).
[1]RC = relative concentration (%); calculated as peak area divided by total peak area. Adj TPC = protein concentration (%) as adjusted for true protein content in the sample. Adj MEC = protein concentration (%) as adjusted for true protein content in the sample and molar extinction coefficient (absorbance) of the protein.
[2]DF treatments of the permeate: RDP = reference; LDP = lactic acid permeate; HDP = HCl permeate; CDP = citric acid permeate; ODP = carbonated permeate.

In the permeate, the total Ca and K were significantly ($P < 0.05$) influenced by some of the acid DF treatments, with CDP having a significantly ($P < 0.05$) higher total Ca content than RDP. In addition, HDP and LDP had a significantly ($P < 0.05$) higher total K content than RDP. The concentration of Ca^{2+} in the permeate was consequently influenced by the acid DF treatment, with HDP, LDP, and ODP showing a significant increase in Ca ions compared with RDP due to the change in pH. The P measured in the permeate most likely represented P_i, as no casein was detected in the DF permeate. The levels of P showed a slight variation between the DF treatments, but no significant differences between the treatments were found. Furthermore, total Mg or Na content in the permeate was significantly ($P < 0.05$) influenced by the DF treatments.

Organic Acids and Lactose in the Retentate and Permeate

As expected, DF with lactic acid and citric acid significantly ($P < 0.05$) increased their contents in their respective retentates and permeates (Table 1), whereas in RDR there was a tendency toward a reduced citric acid content during DF. The lactose content in the retentate seemed to be more reduced within RDR compared with acid DF, whereas its content in the permeate was not significantly ($P < 0.05$) influenced by the various treatments (Table 1).

Rennet Coagulation Properties

The use of acidified DF water significantly ($P < 0.05$) reduced the RCT of the resulting retentate compared with the reference retentate, as shown in Table 4, which was to some extent related to the pH. The LDR obtained the shortest RCT. Neither the firmness of the formed gels nor the curd firming time were significantly ($P < 0.05$) influenced by the treatments.

DISCUSSION

The acid DF step changed the pH of the retentate slightly, as anticipated, whereas the pH of the retentate was only temporarily changed by using only water during DF (RDR). An increase in the retentate pH by addition of DF water has been previously reported (Boiani et al., 2017), as well as a decrease in pH when using acidified DF water. For most of the acid DF retentates, the pH remained relatively stable throughout the DF process, with a tendency for the LDR to further decrease and a tendency for ODR to increase over time during DF. The temperature of the carbonated

Table 4. Effect of acidifying agent used for diafiltration (DF) on the mineral content (m*M*) of the retentate

Sample[1]	Ca	Ca^{2+}	P	P_i[2]	K	Mg	Na
SM	29.74 ± 1.31	1.97 ± 0.16	31.55 ± 2.30	21.92 ± 4.32	44.41 ± 4.29	4.76 ± 0.46	14.37 ± 0.85
MFR	68.65 ± 2.54	2.08 ± 0.19	64.41 ± 1.71	51.81 ± 3.57	48.38 ± 1.34	7.35 ± 0.21	15.71 ± 0.56
RDR	69.43 ± 3.94	2.23 ± 0.48^{a}	64.34 ± 4.13^{b}	52.42 ± 6.51^{b}	40.44 ± 1.30	6.78 ± 0.30^{ab}	13.01 ± 0.28^{ab}
LDR	69.18 ± 4.27	2.64 ± 0.63^{b}	61.89 ± 4.23^{ab}	52.79 ± 7.98^{ab}	40.29 ± 1.31	6.76 ± 0.33^{ab}	12.96 ± 0.19^{a}
HDR	67.59 ± 5.52	2.53 ± 0.47^{bc}	63.09 ± 5.69^{ab}	50.69 ± 9.71^{ab}	41.23 ± 0.77	6.77 ± 0.32^{ab}	13.14 ± 0.24^{ab}
CDR	69.42 ± 1.51	2.30 ± 0.33^{ac}	63.21 ± 2.30^{ab}	51.76 ± 2.74^{ab}	40.42 ± 1.30	6.92 ± 0.21^{b}	13.31 ± 0.46^{b}
ODR	68.13 ± 7.04	2.39 ± 0.19^{abc}	60.61 ± 7.34^{a}	47.54 ± 4.14^{a}	40.16 ± 1.35	6.60 ± 0.45^{a}	12.92 ± 0.46^{a}

$^{a–c}$Means (n = 3 batches) within a column with different superscripts differ according to Tukey's pairwise comparison ($P < 0.05$).

[1]SM = skim milk; MFR = microfiltered retentate. DF treatments of the retentate: RDR = reference; LDR = lactic acid retentate; HDR = HCl retentate; CDR = citric acid retentate; ODR = carbonated retentate.

[2]Inorganic phosphate.

water was low (~4°C) when added to the retentate for DF. The temperature of the retentate, which at that moment was 50°C, automatically dropped together with the pH due to the addition of carbonated water. Throughout the DF process the temperature of the retentate increased to the set DF temperature (50°C). Therefore, the solubility of CO_2 was gradually reduced due to the increase in temperature and equilibration with the atmospheric pCO_2 (pressure change; Hotchkiss et al., 2006).

In concentrated milk, with higher casein and colloidal mineral contents compared with nonconcentrated milk, a shift toward a lower pH value would be necessary for increased solubilization of Ca, P_i, and Mg compared with unconcentrated milk (Le Graët and Gaucheron, 1999). In this study, the protein content of the retentate was 8% and the added acidifiers targeted only a 0.1-unit decrease in pH as the DF was a preparation for cheesemaking. This provided retentates with a final pH ranging from 6.4 to 6.5, and major changes in the mineral balance were not expected, resulting in a nonsignificant change in total Ca, and changes in total P, P_i, and Mg for only some acid DF (CDR and ODR) compared with RDR. The total and soluble Ca contents in the permeate, however, reflected the influence of acid DF and indicated a trend toward their increase in the acidified permeates compared with the reference (RDP). Citric acid treatment expressed the most significant increase in soluble Ca content of the permeate. These results are consistent with the findings of Kulozik (1998) showing only minimal changes in micellar Ca levels during DF despite the loss of soluble Ca.

The reduction of the pH in milk caused by acidification is usually coupled to an increase in the ionic Ca content (Le Graët and Gaucheron, 1999). However, the Ca^{2+} content of the retentates in this experiment is attributed more to the type of acidifying agent used during DF and their affinity to bind Ca^{2+}. de Kort et al. (2011) reported a decrease in Ca ion activity upon addition of citrate to a concentrated micellar casein solution when studying the effect of citrate on the physical changes in casein micelles in a concentrated micellar casein solution; this is due to the high Ca-chelating ability of citrate. The use of citric acid with DF, therefore, is expected to shift the Ca equilibrium toward soluble Ca citrate chelates (Mizuno and Lucey, 2007). The high affinity of citrate to Ca ions was also reflected on the total Ca content in the permeate, which was higher than the reference (RDP).

Guillaume et al. (2004) explained that in skim milk depressurized after CO_2 addition, the mineral composition is restored to its colloidal state. The influence of the solubilization by CO_2 on the CCP is reversible at pH values above 5.8, with no insoluble forms of salt formed, after which Ca binds back to P_i and shifts back into the micelle. The observed significant increase in Ca^{2+} in the ODP compared with the reference (RDP) may seem contradictory to the abovementioned results. This phenomenon is explained by the fast and continuous removal of Ca^{2+} toward the permeate during the DF process while the depressurization of CO_2 from the retentate is not yet fully complete or established, leading to a significantly ($P < 0.05$) higher Ca^{2+} content in the permeate compared with the reference.

The acid DF permeate and retentate might have formed other compounds that were not measured and could contribute to the explanation for the compositional changes occurring between the retentate and permeate phases. The hydrocarbonate (HCO_3^-), in the case of DF with CO_2 for example, can easily bind to solubilized Ca and Mg and form carbonates.

The distribution of minerals between the permeate and retentate, as influenced by the combined DF and addition of acidifiers, is summarized in Figure 4. The MF process increases the micellar Ca and P contents in the retentate as a result of the concentration of casein micelles and the removal of milk serum. The type of DF medium influences the equilibrium of cations, anions,

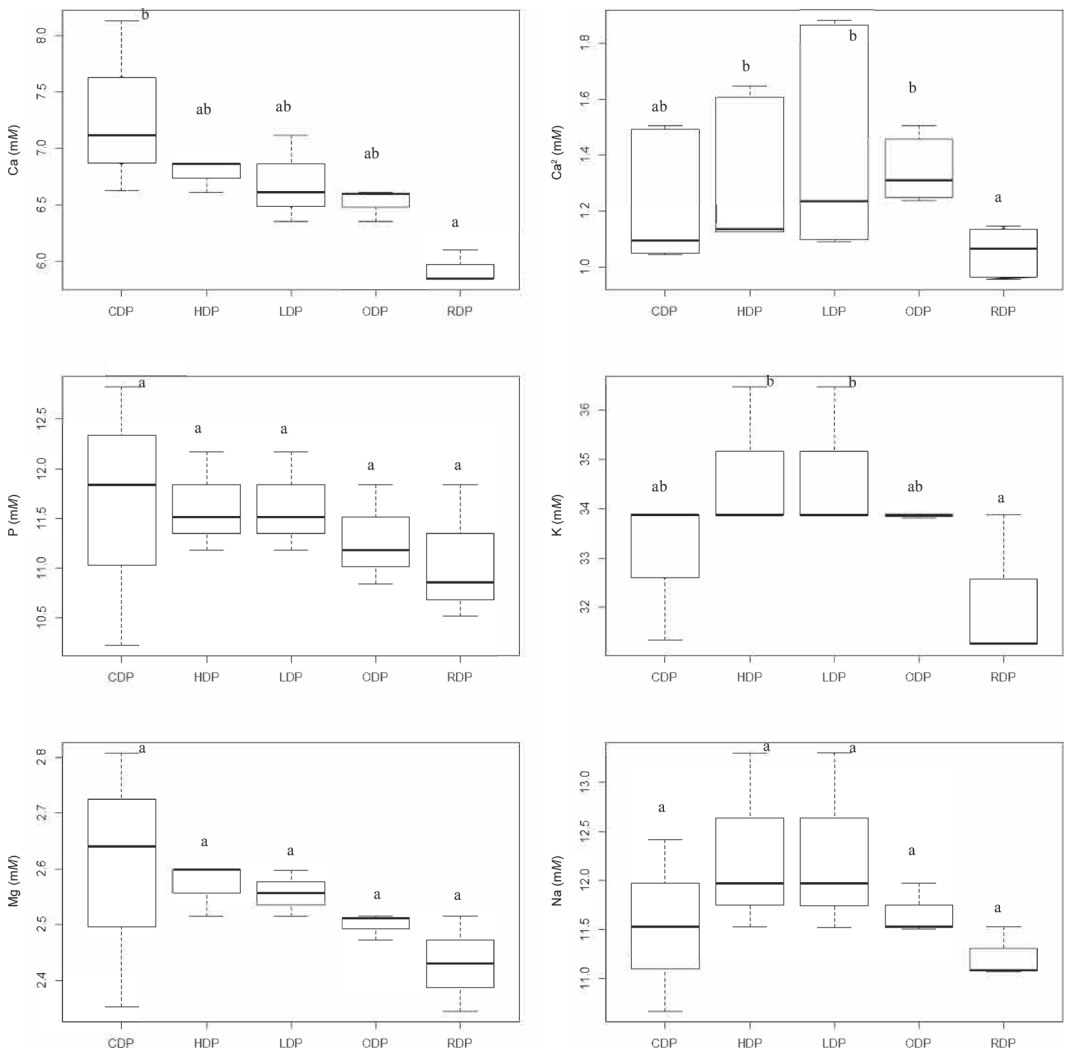

Figure 3. Mineral content (m*M*) of permeate from the diafiltration process of microfiltered retentate. CDP = citric acid permeate; HDP = HCl permeate; LDP = lactic acid permeate; ODP = carbonated permeate; RDP = reference. Means and SD of 3 batches. Letters (a, b) within the same plot represent significance differences ($P < 0.05$) between the diafiltration treatments. Boxes describe the variations in the data, mid lines are the median that splits the data between the lower 50% of observation from the upper 50% of observation, the lower whisker is the minimum and represents the lowest observation, and the upper whisker is the maximum and represents the highest observation in the dataset.

and soluble salts within the soluble or micellar phase of the retentate, which are then able to permeate the membrane or remain in the retentate.

The RCT is mostly correlated with the pH value and available Ca^{2+} content (Dalgleish and Corredig, 2012): the lower the pH and the higher the Ca^{2+} concentration, the shorter the RCT. Lactic acid diafiltered retentate having the highest Ca^{2+} content and the lowest pH value explains the shortest RCT. A similar phenomenon was observed by Calvo et al. (1993) when comparing the RCT of lactic acid versus CO_2-treated milk during cheesemaking. Lactic acid has the ability to form more soluble Ca^{2+} compared with CO_2 and citric acid. The latter 2 form Ca carbonates and Ca citrate, respectively, which have lower solubility. Guillaume et al. (2004) suggested that CO_2 follows a mechanism that involves a change in the form of CCP and reorganization of the casein micelle and its surface activity in addition to the Ca^{2+} levels. They also reported that only carbonation to a pH below 5.8 improved the rheological properties of renneted gels, which is in line with the current finding showing that the different DF treatments did not influence the gel firmness compared with the reference. Despite the lack of effect by acid DF on curd firmness, it is important to consider the potential contribution of the slightly lower pH and change in Ca^{2+} to the further development of texture during cheesemaking due to the level of protein solvation (Lawrence et al., 1987).

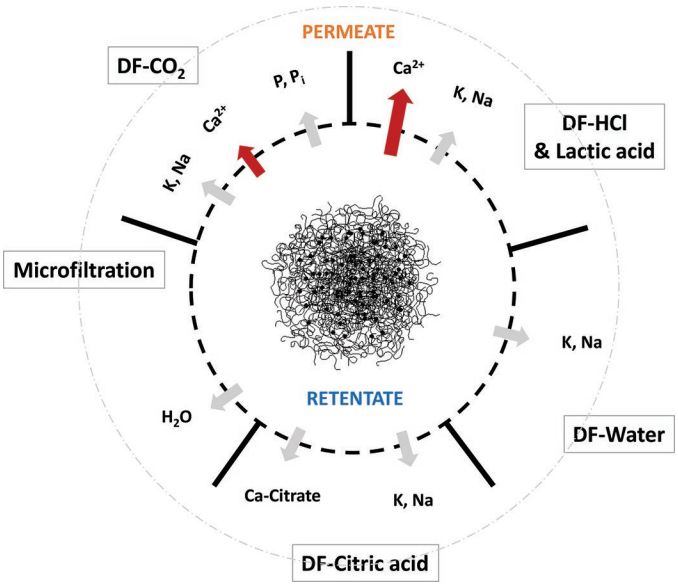

Figure 4. Schematic representation of the distribution of minerals between the permeate and retentate as influenced by the decrease in pH due to addition of acidifiers. DF = diafiltration.

Another consideration would be the significantly ($P < 0.05$) higher levels of lactic and citric acid in the LDR and CDR, respectively, which may influence the cheese composition and texture characteristics. Citric acid can contribute to soft curd during cheesemaking (Shehata et al., 1967), and milk acidified with CO_2 has been reported to negatively influence the proteolytic and lipolytic activities of the starter culture and thereby cheese ripening (MacCarney et al., 1995). An important note is that despite the higher citric acid content in the CDR samples compared with the other DF treatments, the level of citric acid was similar to the original content in the skim milk, which might be of importance in cheese varieties in which the degradation of citrate to CO_2 and diacetyl is important for cheese ripening.

The apparent stability in lactose content of the retentate following acid DF is similar to findings reported by Caron et al. (1997), who recorded the same lactose concentration between retentate powders produced by regular DF-MF or lactic acid-acidified MF-DF retentate. However, a tendency toward an increased retention of lactose in the acid DF retentate was observed in this study compared with the nonacid DF retentate. The use of a 0.3-DV DF avoided disruption to the casein micelles, as findings by Ferrer et al. (2014) and Boiani et al. (2017) showed casein micelle disruption at a DV of 0.5 and above. The absence of caseins in the permeate and their nonsignificant change in the retentate attain that. The WP content, however, was influenced in both the retentate and the permeate following acid DF. The decrease in total WP content of the acid DF retentate has been previously reported by Caron et al. (1997). In the present study, HDR and ODR had the lowest WP content compared with the reference.

Protein analysis of the permeate showed that DF water had an enhanced ability to transfer more WP to the permeate than acid DF water with lactic acid and CO_2. The reduced total WP content in LDP and ODP was reflected by the reduced concentration of α-LA and β-LG compared with RDP. This result could be explained by the ability of dissolved CO_2 to react with the amine groups of protein, forming carbamate (Jones and Greenfield, 1982). This study showed that the transfer of WP to the permeate was altered by the addition of acidifiers to the DF water.

CONCLUSIONS

This study showed that the added acidifiers (lactic acid, HCl, citric acid, and CO_2) changed the retentate and permeate composition after acid DF of MF casein concentrates. This was observed even at a small pH reduction of 0.1 unit and a relatively small DF factor. The most noticeable change in the retentate was an increase in Ca^{2+} content and reduced clotting time when using lactic acid and HCl to acidify the DF water. The permeate issued from acid DF had an overall reduced WP content compared with the DF with only water as well as an increased mineral content. Citric acid presented the highest affinity to bind Ca, with a significant increase in total Ca content in the permeate and lower Ca^{2+} content in the retentate and permeate. Each acidifying agent had slightly different effects on the retentate and permeate composition. The choice of acidifier in the DF process should be considered based on its positive contribution to the textural attribute of the desired dairy product or the quality of the sidestream permeate.

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