International Dairy Journal 95 (2019) 6–14

Contents lists available at ScienceDirect

International Dairy Journal

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

Improving rennet coagulation and cheesemaking properties of reverse osmosis skim milk concentrates by pH adjustment

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ARTICLE INFO

Article history:
Received 29 November 2018
Received in revised form
21 February 2019
Accepted 21 March 2019
Available online 30 March 2019

ABSTRACT

The use of reverse osmosis (RO) for cheese milk concentration has advantages including obtaining reusable low pollutant permeates and reducing milk transportation costs. However, high levels of lactose and salts in RO concentrates impair their cheesemaking abilities. The objective of this work was to optimise the use of RO concentrates (5–11% protein content) for cheesemaking by pH adjustment. Rennet coagulation kinetics, salt partitioning and cheesemaking properties were studied in comparison with ultrafiltration concentrates. Results showed that concentration by RO induced an increase regarding the coagulation time and the gel maximal firming rate that reached a plateau at 9% protein content. Increases in calcium mineralisation of casein micelles as well as in yield, moisture and lactose content in model cheese were observed. Lowering renneting pH was found to improve the cheesemaking properties of RO concentrates by promoting partial demineralisation of casein micelles, accelerating coagulation kinetics and increasing curd drainage.

1. Introduction

The cheese manufacturing industry has increasing interests in concentrating milk prior to cheesemaking to achieve benefits in yield and production efficiency (Hydamaka, Wilbey, & Lewis, 2000). Ultrafiltration (UF) is currently the preferred membrane process for pre-concentration of cheese milk (Mistry & Maubois, 2017). However, reverse osmosis (RO), which is widely used for processing whey, also has potential to be used in cheese production. It is a pressure-driven membrane process similar to evaporation, as it concentrates all the milk constituents while only water and trace amount of low molecular weight components are removed. RO concentrates have the potential to increase the recovery of lactose and minerals in cheese with a significant impact on cheese yield. The main advantage of RO over evaporation is the lower energy consumption (Hiddink, de Boer, & Nooy, 1980). RO concentration can be handled as a way to produce water from dairy fluids that can be reused in dairy processing plants for diafiltration or a cleaning operation after a polishing step (Balannec, Gésan-Guiziou, Chaufer, Rabiller-Baudry, & Daufin, 2002). Also, on-farm concentration of milk may reduce the costs and the environmental impacts related to its transportation (Cox & Langdon, 1985; Wenten & Khoiruddin, 2016).

RO concentrates are characterised by higher viscosity because of higher lactose and salt contents compared with UF concentrates and skim milk (Lauzin, Dussault-Chouinard, Britten, & Pouliot, 2018). Concentration by RO is expected to induce a shift from soluble to colloidal calcium, magnesium and phosphorus, causing an increase in mineralisation of casein micelles (Le Graët & Brule, 1982). A change in the salt balance is critical in the cheesemaking process (De La Fuente, 1998) and greatly influences the rennet coagulation of casein micelles. This initial step of cheesemaking is known to depend on pH, calcium concentration, protein content and calcium associated with casein (Lucey, Johnson, & Horne, 2003; Soodam & Guinee, 2018). The rennet coagulation kinetics of RO concentrates were found to be slowed, with an increased rennet coagulation time and a lower maximal firming rate compared with UF ones (Lauzin et al., 2018). The final yield and composition of cheese are affected by the coagulation properties and curd firmness. Gel firmness at cutting has been shown to affect cheese moisture as well as fat and protein recovery (Fagan, Castillo, Payne, O'Donnell, & O'Callaghan, 2007).

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https://doi.org/10.1016/j.idairyj.2019.03.008
0958-6946/

Some attempts have been made to produce cheeses from RO concentrated milk. Agbevavi, Rouleau, and Mayer (1983) prepared Cheddar from whole milk concentrated twofold. The composition of cheese made from RO-concentrated milk was close to that of traditional Cheddar, but it had higher lactose concentration and a non-uniform granular texture. Barbano and Bynum (1984) and Bynum and Barbano (1985) studied the yield and composition of cheeses made from RO concentrated milks with 12%–15% solids content. The authors observed higher lactose content in cheese made from RO milk (15% solid content) than in the control cheese and an increase in cheese yield by 2–3% above expected theoretical yields. Despite the positive impact on yields, high lactose concentration in cheese can induce quality defects during ripening due to post-acidification phenomena (Bynum & Barbano, 1985). The use of RO milk may then be restricted to cheese with a very short maturation period or should be limited to low concentration factors (Bynum & Barbano, 1985).

Lauzin et al. (2018) also studied the cheesemaking properties of milk concentrates (22% solids content). Model cheeses made from RO milk were characterised by higher moisture-adjusted yield and higher moisture content than cheese made from control skim milk. Some plants already use a thermal evaporator to pre-concentrate cheese milk (Honer, 1984) and excessive lactose and salts in the curd mass are removed by washing with water during cheesemaking (Mistry & Maubois, 2017). The high content of both lactose and salts in RO concentrates seems to remain the biggest concern about using this process to pre-concentrate cheese milk because it impairs coagulation and cheese properties.

The use of UF for concentrating milk prior to cheesemaking has been extensively studied (Mistry & Maubois, 2017). UF is a pressure-driven membrane process that concentrates the protein fraction and colloidal salts of milk, while the composition of the soluble phase remains constant. The use of UF as pre-concentration of cheese milk leads to an increase of both the gel-firming rate and the cheese yield. It also has little effect on cheese composition with, moisture generally decreasing with increasing milk protein concentration (Guinee, O'Kennedy, & Kelly, 2006; Guinee, Pudja, & Mulholland, 1994).

To improve the use of RO concentrates for cheesemaking and to obtain a good quality cheese, a correction regarding physico-chemical properties needs to be done before the coagulation step. A decrease in pH is known to promote the solubilisation of colloidal salts and to reduce the electrostatic repulsion between casein micelles. In milk, the colloidal phosphate and associated calcium completely solubilise at pH 5.20 (Gaucheron, Le Graët, & Schuck, 2003). Both the hydrolysis and the aggregation phases of enzymatic coagulation are affected by a pH adjustment. The rennet activity and the gel-firming rate are known to be increased by a lower pH (Karlsson, Ipsen, & Ardö, 2007a). Thus, the high mineralisation of casein micelles and impaired coagulation kinetics could be corrected by a pH adjustment of RO concentrates. As the amplitude of physico-chemical alterations are also dependant on the milk concentration, it is pertinent to study different concentration factors to determine the concentration limit for making good quality cheese. For each RO concentration, it appears relevant to find the pH that leads to the appropriate mineralisation of casein micelles before cheesemaking. Consequently, the aim of this study was to investigate the effect of a pH adjustment regarding RO concentrates (5–11.0% protein content) on the salt partitioning and the kinetics of rennet coagulation. Model cheeses were also made to characterise the effect of acidification and protein content of concentrates on the cheese yield, composition and rheology. UF concentrates with the same protein content as the RO concentrates were used as controls in this study.

2. Materials and methods

2.1. Skim milk supply

Pasteurised skim milk was obtained from a local dairy supplier and stored at 4 °C for a maximum of two days until experiments. For each of the three replicates, the same batch of milk was used for both UF and RO concentration.

2.2. Preparation of UF and RO concentrates

The concentration process was carried out using a pilot filtration unit (Model 1812 Lab Unit, Filtration Engineering Company Inc., Champlin, MN, USA) equipped with a 0.32 m^2 spiral-wound membrane. UF concentration was processed with a polyethersulfone membrane (model ST3B-1812F, Synder Filtration, Vacaville, CA, USA) with a cut-off of 10,000 Da. For the RO process, the membrane was made of polyamide and had a 99% average NaCl rejection (Model AG1812C 34B, General Electric, Trevose, PA, USA). Inlet pressure of UF and RO processes were respectively 4 bars and 20.6 bars. Skim milk was heated to 50 °C prior to concentration, and this temperature was kept constant during the filtration. The protein content of skim milk was analysed before filtration by the total nitrogen Kjeldahl method and calculated using 6.38 as the conversion factor. For both UF and RO filtration, the concentration was stopped when the retentate reached a protein content of 11.2%, as measured by the Dumas method (TruSpec, Leco®, St. Joseph, MI, USA). It was then diluted back to 11%, 9%, 7% and 5% protein content with the same permeate that was obtained from filtration. Protein content of the resulting concentrated milk was also tested by Dumas method for confirmation. Three repetitions of RO and UF processes were done with three different batches of milk.

2.3. pH adjustment

Skim milk and UF retentates were used as controls and their pH were adjusted to 6.50. Each RO retentate (5, 7, 9 and 11% protein content) was adjusted to four levels of pH (6.50, 6.20, 5.90 and 5.60) for a total of 16 combinations. It was hypothesised that this range of pH would promote an adequate demineralisation of casein micelles. The pH adjustment was made by adding 21% (w/w) lactic acid (Thermo Fisher Scientific, Waltham, MA, USA) or 1 M NaOH (Thermo Fisher Scientific) while stirring the samples with a magnetic bar to avoid local precipitation. It was monitored with a SympHony pH meter (VWR Scientific, Radnor, PA, USA). Retentates were left to equilibrate overnight (18 h) at 4 °C. Before further analysis, concentrated milks were held at 32 °C for 1 h, and then pH was adjusted if necessary.

2.4. Soluble-colloidal equilibrium and composition of retentates

Soluble and colloidal phases of UF and RO retentates were separated by ultracentrifugation (Lauzin et al., 2018; Liu, Dunstan, & Martin, 2012) at 100,000× *g* for 60 min at 32 °C (Optima XE-90 Ultracentrifuge, Beckman Coulter, Brea, CA, USA). The soluble phase was defined as the one that did not sediment after ultracentrifugation. Salts content (Ca, Mg, K, Na and P) of concentrates and their soluble fractions was measured by inductively coupled plasma optical emission spectroscopy (ICP-OES) (Optima 4300 DV, Perkin–Elmer, Waltham, MA, USA). Ashes were prepared by sample calcination overnight at 550 °C (Thermolyne Furnace with Furnatrol 1; Thermo Fisher Scientific). Ashes were then collected in 3 mL of glacial tricholoroacetic acid (TCA) 20% (w/v) (Anachemia, Radnor, PA, USA) and diluted up to 50 g in Milli-Q water. The solution was

then vortexed and passed through a 0.45 μm filter (Starstedt, Nümbrecht, Germany) before analysis.

The salts were analysed in triplicate. The nitrogen content (total nitrogen [TN], non-casein nitrogen [NCN], and non-protein nitrogen [NPN]) of the concentrates and their soluble fractions was quantified by the Kjeldahl method (AOAC, 2000) (procedures 991.20, 998.05, and 991.21 respectively). Total protein content was calculated by multiplying TN by a factor of 6.38. True protein was determined as (TN-NPN) × 6.38, and casein as (TN-NCN) × 6.38. Protein analyses were made in duplicate. The total solids measurements of concentrates were obtained by gravimetry after dehydration in a forced air oven at 100 °C for 4 h (AOAC 990.20). The lactose content of concentrated milk was determined by high-performance liquid chromatography (HPLC) with a Waters chromatograph (Waters Corp., Milford, MA, USA), equipped with a Hitachi (Foster City, CA, USA) differential refractometer detector L-7490, a 600E controller, a column oven and a cooled 717 Plus autosampler. An ICSep ICE-ION-300 column (Transgenomic, Omaha, NE, USA) was used with 8.5 mM H_2SO_4 (180 H_2SO_4 L^{-1}) as the mobile phase at a flow rate of 0.4 mL min^{-1}. The column temperature was kept constant at 40 °C. Samples were diluted in ultrapure water and passed through a 0.45-μm nylon filter (Chromatographic Specialties, Brockville, ON, Canada) before injection (15 μL), according to the reference method (ISO 22662). An anhydrous lactose (Sigma PHR1025) preparation was used as an external standard to perform quantification (linear from 50 ppm to 10,000 ppm). The run time was 45 min.

2.5. Rennet coagulation kinetics

The rennet used was the ChymO-PLUS I200PRE031 (Fromagex, Rimouski, QC, Canada) with an average activity of 650 international milk-clotting units (IMCU) mL^{-1}. It was diluted 1:10 in Milli-Q water and added within 5 mi to milk. Twenty-five microlitres of diluted rennet was added to 25 mL of concentrated milk to achieve a 0.01% final concentration (0.065 IMCU mL^{-1}). In our study, rennet was added to milk and concentrated milk at a constant volume concentration as reported in many experiments (Lauzin et al., 2018; Sandra, Cooper, Alexander, & Corredig, 2011; Waungana, Singh, & Bennett, 1998; Zhao & Corredig, 2016). Milk samples were stirred for 30 s after rennet addition.

Rheological measurements were made using a stress-controlled rheometer (ARES-G2, TA Instruments, New Castle, DE, USA) equipped with concentric cylinders (27.7 and 30 mm in diameter, also from the same supplier). A Peltier temperature control system (C-PTD 200, TA Instruments, New Castle, DE, USA) kept the setting to 32 °C all along measures taken. A 25 mL aliquot, to which rennet had been added, was poured in the cup of the rheometer and the test was started exactly 2 min after rennet addition. The storage modulus (G′) was recorded with TRIOS software (v3.3.1.4668, TA Instruments, New Castle, DE, USA). A constant strain value of 0.1% was applied at a frequency of 1 Hz (Perreault, Turcotte, Morin, Pouliot, & Britten, 2016). Rennet coagulation time (RCT) was defined as the period until G′ increased by 1 Pa. The maximal firming rate (MFR) of the gel was calculated from the first derivative of the slope where G′ varies with time and corresponds to the coagulation rate (slope) at the inflection point. Values of G′ at 60 min was also extracted from the data.

2.6. Model cheese production

Concentrated skim milks samples for cheesemaking were prepared as described in section 2.2. For each milk protein concentration (5, 7, 9 and 11%), three types of cheese were produced. First, UF control cheeses were made from milk concentrated by UF and adjusted to pH 6.20 before renneting. A second type of cheese was made from milk concentrated by RO and adjusted to pH 6.20, which corresponds to the usual value for drainage in Cheddar production (Perreault et al., 2016). Since lactic acid was used for acidification instead of lactic acid bacteria, the pH of 6.20 at renneting was used instead of 6.50 to reproduce the decrease of pH that usually occurs during cheesemaking.

A third type of cheese was made from milk concentrated by RO and highly acidified before renneting. The renneting pH of those milk concentrates was defined as the pH needed to reach the same colloidal calcium mineralisation of casein micelles as those of UF concentrates. Those pH values were determined from salt-partitioning results and had also been decreased by 0.3 pH unit before renneting (5%, pH 5.75; 7%, pH 5.70; 9%, pH 5.45; 11%, pH 5.30) to reflect the decrease of pH that usually occurs during cheesemaking.

Finally, cheese was made from unconcentrated skim milk adjusted to pH 6.20. Model cheeses were made with some adjustments to the method described by Morin, Pouliot, and Britten (2008). Briefly, 145 g milk was tempered at 32 °C for 60 min and then renneted (0.01% rennet concentration). Then, 144 g of renneted milk was poured into a 12 × 12 cm vat with plexiglass covers layered with cheesecloth placed inside an orbital shaking water bath (OLS 200; Grant Instruments, Cambridge, UK). The renneted milk was left without agitation at 32 °C until the cutting time was reached. The latter was defined as the period needed for the gels to reach a fixed strength (G′) of 40 Pa, as measured by dynamic rheology. The curd was cut with a stainless steel knife to obtain 1 cm^3 cubes and left without agitation for 2 min. The motion of the orbital shaking water bath was set to 40 rpm for 10 min after which the temperature was adjusted to 34 °C and gradually raised to 42 °C in 40 min (1 °C per 5 min) while shaking at 60 rpm. The curd was first drained by reversing the cheese vat for 30 min. It was then transferred in two 50 mL plastic centrifuge tubes. The bottom conical part of each one was filled with resin as described in Perreault et al. (2017). The centrifugation was performed at 4400× *g* for 60 min at 32 °C using a 5804 R Centrifuge (Eppendorf AG, Hamburg, Germany) with a swinging bucket rotor A-4-44. The resulting whey was collected and added to whey from drainage of the curd. Cheese and whey were weighed and kept at 4 °C until analysis. Cheeses were vacuum-packed in plastic bags.

2.7. Cheese composition and rheological analysis

The actual yield of cheese production was calculated using equation (1):

$$Actual\ yield(\%) = (mass\ of\ curd/mass\ of\ milk)*100 \quad (1)$$

The actual yield was adjusted to constant moisture (60%) using equation (2):

$$Moisture\ adjusted\ yield\ (\%) = actual\ yield* (1-(CM/WM))/(1-(60/WM)) \quad (2)$$

where *CM* = curd moisture (%) and *WM* = whey moisture (%)

Curd moisture was determined by gravimetry after dehydration of the crushed curd in a forced air oven at 100 °C for 5 h (AOAC 990.20). A whole cylindrical cheese was used for this analysis and the second one for rheological measurement, as described below. Milk and whey were analysed for protein, lactose and calcium contents. Cheese composition was obtained through mass balance calculations.

Protein retention was calculated using equation (3):

$$True\ protein\ retention(\%) = 100*(1 - [\%true\ protein\ in\ whey]/[\%\ true\ protein\ in\ milk]) \quad (3)$$

Seven days after the production, cheeses were analysed for their rheological properties. A frequency sweep was performed using a stress-controlled rheometer (ARES-G2, TA Instruments, New Castle, DE, USA) equipped with 25 mm serrated parallel plates for geometry (TA Instruments). For each experimental cheese, three discs (25 mm in diameter and 3 mm in height) had been sampled with a cylindrical guide and cutting wire. These were cut: (i) at 3 mm from the bottom end; (ii) in the middle; and (iii) at 3 mm from the top end of the cheese. The frequency sweep was realised on the three discs according to the method of Perreault et al. (2017). Briefly, each one was compressed at 1 N for 5 min at 20 °C before processing it. Constant strain amplitude (0.1%) was applied to the sample, and the oscillation frequency was increased from 0.5 Hz to 10 Hz. The complex modulus (G*) and the phase angle (δ) were recorded as a function of the oscillation frequency and fitted to a power law model (equations (4) and (5)):

$$G^* = G_1^* \omega^{G^* fd}, \quad (4)$$

$$\delta = \delta_1 \omega^{\delta fd}, \quad (5)$$

where G^*_1 and δ_1 represented respectively the complex modulus and phase angle measured at 1 Hz and ω was the frequency (in hertz). G^*_{fd} and δ_{fd} were the power indices, which represent the frequency dependence of the moduli between 0.5 Hz and 10 Hz (Banville, Morin, Pouliot, & Britten, 2014).

2.8. Statistical analysis

The whole experiment was repeated three times, and all analyses were performed at least in duplicate. Data were analysed as a split-plot design. Analysis of variance and Tukey's honestly significant difference test were carried out on experimental measurements with 95% confidence level using (SAS Institute Inc., Cary, NC, USA). Differences were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Milk composition as a function of the concentration

Table 1 shows the overall composition and salt partitioning of UF and RO concentrates at pH 6.50 as a function of the concentration. The concentration of both UF and RO concentrates is expressed in terms of true protein content and varies from 3.22% (skim milk) to 10.7%. The concentration by UF did not significantly ($P > 0.05$) affect the initial pH of the concentrates, which is consistent with the results of Sandra et al. (2011). However, for RO concentration, the initial pH decreased as the concentration factor increased, with values varying from 6.61 (skim milk) to 6.28 (RO, 11% protein content). As this concentration proceeded, the precipitation of phosphate calcium salts induces the dissociation of phosphoric acid, which liberates protons and decreases the resulting pH (Gaucheron, 2005; Lauzin et al., 2018; Syrios, Faka, Grandison, & Lewis, 2011). The pH of concentrates had been adjusted to 6.50 before further analysis. The composition and salt content regarding the control skim milk were similar to values found in literature (Gaucheron, 2005; Syrios et al., 2011). It had a colloidal calcium/colloidal casein ratio of 30.89 mg g^{-1}, which is similar to the ratio of 30.86 mg g^{-1} found by Gaucheron (2005). Concerning the concentration by UF, the total content of protein, calcium, phosphorus and magnesium increased, while the content of lactose and monovalent ions (sodium and potassium) remained unchanged ($P > 0.05$) compared with the control milk. As expected, RO concentrates showed a proportional and significant increase ($P < 0.05$)

Table 1
Mean composition and salt partitioning of skim milk, RO concentrates and UF concentrates (expressed in terms of protein content) adjusted to pH 6.50.[a]

Parameter	Skim milk	Protein content of RO concentrates				Protein content of UF concentrates			
		5%	7%	9%	11%	5%	7%	9%	11%
Initial pH at 32 °C	6.61 ± 0.04^{d}	6.45 ± 0.02^{c}	6.36 ± 0.03^{b}	6.32 ± 0.03^{ab}	6.28 ± 0.01^{a}	6.60 ± 0.03^{d}	6.58 ± 0.03^{d}	6.58 ± 0.04^{d}	6.56 ± 0.05^{d}
Total solids (g 100 g^{-1})	8.72 ± 0.14^{a}	13.18 ± 0.17^{c}	18.22 ± 0.23^{d}	23.42 ± 0.39^{e}	29.56 ± 0.53^{f}	11.14 ± 0.50^{b}	13.54 ± 0.94^{c}	16.18 ± 1.38^{d}	18.79 ± 1.97^{d}
True protein (g 100 g^{-1})	3.22 ± 0.01^{a}	4.81 ± 0.29^{b}	6.78 ± 0.24^{c}	9.05 ± 0.06^{d}	10.7 ± 0.4^{e}	4.89 ± 0.03^{b}	6.84 ± 0.27^{c}	8.97 ± 0.32^{d}	10.70 ± 0.35^{e}
Casein (g 100 g^{-1})	2.70 ± 0.04^{a}	4.06 ± 0.28^{b}	5.76 ± 0.27^{c}	7.70 ± 0.10^{d}	9.00 ± 0.51^{e}	4.16 ± 0.04^{b}	5.79 ± 0.19^{c}	7.63 ± 0.22^{d}	9.14 ± 0.30^{e}
Soluble casein (g 100 g^{-1})	0.09 ± 0.01^{a}	0.18 ± 0.02^{b}	0.23 ± 0.02^{bc}	0.34 ± 0.03^{cde}	0.36 ± 0.06^{de}	0.22 ± 0.00^{bc}	0.27 ± 0.06^{bcd}	0.37 ± 0.06^{de}	0.40 ± 0.03^{e}
Ratio soluble/total casein (%)	3.35 ± 0.24^{a}	4.53 ± 0.39^{bc}	4.07 ± 0.28^{b}	4.42 ± 0.41^{bc}	4.03 ± 0.93^{b}	5.40 ± 0.21^{c}	4.73 ± 0.81^{bc}	4.83 ± 0.70^{bc}	4.35 ± 0.39^{b}
Lactose content (g 100 g^{-1})	4.79 ± 0.08^{a}	7.14 ± 0.14^{b}	9.94 ± 0.36^{c}	12.69 ± 0.13^{d}	15.69 ± 0.49^{e}	4.64 ± 0.09^{a}	4.75 ± 0.04^{a}	4.65 ± 0.31^{a}	4.93 ± 0.06^{a}
Ash content (g 100 g^{-1})	0.76 ± 0.02^{a}	1.22 ± 0.08^{cd}	1.66 ± 0.03^{f}	2.13 ± 0.04^{g}	2.34 ± 0.03^{h}	0.95 ± 0.02^{b}	1.14 ± 0.07^{c}	1.35 ± 0.07^{de}	1.51 ± 0.09^{ef}
K (mM)	33.94 ± 0.73^{a}	58.35 ± 3.33^{b}	82.20 ± 3.00^{c}	112.9 ± 6.1^{d}	133.2 ± 8.8^{e}	37.31 ± 4.44^{a}	39.38 ± 2.55^{a}	43.24 ± 4.77^{a}	45.61 ± 2.63^{ab}
Na (mM)	19.43 ± 0.24^{a}	29.48 ± 4.05^{ab}	41.80 ± 3.69^{b}	58.82 ± 5.77^{c}	68.90 ± 7.84^{c}	20.84 ± 1.28^{a}	25.73 ± 2.57^{a}	26.85 ± 3.13^{a}	25.50 ± 2.09^{a}
Mg (mM)	4.18 ± 0.08^{a}	6.88 ± 0.51^{bc}	9.63 ± 0.42^{cd}	13.06 ± 1.17^{e}	15.38 ± 1.64^{f}	6.22 ± 0.61^{b}	7.93 ± 1.33^{bcd}	9.39 ± 1.33^{cd}	10.00 ± 0.98^{de}
Ca (mM)	29.01 ± 0.5^{a}	44.91 ± 3.70^{b}	63.14 ± 2.96^{c}	89.62 ± 6.19^{d}	104.73 ± 6.74^{e}	42.09 ± 3.53^{b}	60.02 ± 5.58^{c}	76.35 ± 3.29^{cd}	87.64 ± 5.07^{de}
P (mM)	34.67 ± 0.55^{a}	46.70 ± 3.20^{bc}	65.64 ± 2.43^{de}	91.26 ± 6.76^{f}	105.40 ± 8.77^{g}	39.61 ± 3.64^{b}	54.79 ± 4.39^{cd}	66.70 ± 2.80^{de}	74.95 ± 2.81^{ef}
Ratio soluble/total Ca (%)	34.42 ± 0.88^{f}	18.69 ± 0.67^{d}	14.66 ± 0.23^{c}	9.95 ± 1.06^{a}	8.17 ± 0.57^{a}	21.48 ± 0.63^{e}	15.83 ± 0.05^{c}	12.32 ± 1.36^{b}	9.37 ± 0.26^{a}
Ratio coll. Ca/coll. casein (g 100 g^{-1})	30.89 ± 0.67^{a}	37.40 ± 1.14^{c}	38.67 ± 1.24^{c}	42.00 ± 0.30^{d}	42.19 ± 0.24^{d}	33.63 ± 1.28^{b}	35.94 ± 1.43^{bc}	36.29 ± 1.11^{bc}	35.66 ± 1.75^{bc}
Ratio soluble/total P (%)	39.51 ± 0.69^{g}	30.69 ± 0.41^{f}	26.62 ± 1.23^{e}	19.62 ± 1.64^{cd}	16.29 ± 1.25^{b}	30.45 ± 1.00^{f}	22.58 ± 0.75^{d}	17.31 ± 1.23^{bc}	12.98 ± 0.35^{a}
Ratio coll. P/coll. casein (g 100 g^{-1})	23.39 ± 0.60^{ab}	24.76 ± 1.39^{b}	25.15 ± 1.36^{b}	28.41 ± 0.38^{c}	29.66 ± 0.95^{c}	21.13 ± 1.80^{a}	22.82 ± 1.30^{ab}	22.81 ± 0.37^{ab}	22.35 ± 0.66^{ab}

[a] Data are based on triplicate experiments; values in the same row without a common superscript letter are significantly different (Tukey, $P < 0.05$).

of all milk constituents (protein, salt and lactose) as the concentration proceeded.

In the case of UF concentration, the total calcium increase was less pronounced than that for the protein, with concentration factors varying from 1.45 to 3.02 for the total calcium and from 1.52 to 3.32 for the true protein, because substantial amount of calcium is removed in the permeate (Syrios et al., 2011). The colloidal calcium/colloidal casein ratio regarding UF concentrates, which is an indicator of the casein mineralisation, was not significantly affected ($P > 0.05$) by the increase of the concentration; similar results were previously reported by Sandra et al. (2011). As the colloidal calcium was retained by the casein micelles and a part of the soluble calcium was allowed to flow through the membrane, the soluble/total calcium ratio decreased as the concentration increased.

The RO concentration of milk strongly affected the salt balance between the soluble and the colloidal phases, with an increase in the colloidal calcium/colloidal casein ratio from 30.89 mg g^{-1} (skim milk) to 42.19 mg g^{-1} (RO, 11%). The same tendency was observed for phosphorus. As the amount of calcium associated with casein micelles increased, the soluble/total calcium ratio decreased. These results are consistent with those found by Liu et al. (2012) and Le Graët and Brule (1982), where concentration was being achieved by evaporation under vacuum at 50 °C instead of RO. Liu et al. (2012) explained this shift of soluble calcium from the serum to the micelles by the decrease in solubility of calcium and phosphorus when water is removed. The temperature of the filtration (50 °C) can also lower the solubility of salts. However, the dynamic equilibrium is rapidly reversible (De La Fuente, 1998) when milk is warmed up to 32 °C before analysis. It is still not clear how the salts are transferred to the micelles and whether or not they associate with existing colloidal calcium phosphate nanoclusters (Liu et al., 2012). Some authors reported that, as a consequence of RO concentration, the pH of concentrates decreases and the ionic strength increases, both factors reducing the calcium mineralisation of casein micelles (Snoeren, Brinkhuis, Damman, & Klok, 1984). In our study, this effect was absent because of the pH adjustment of concentrates at pH 6.50.

3.2. Casein mineralisation as a function of the pH

The calcium mineralisation of casein micelles as a function of the pH and the concentration by RO is presented in Fig. 1. RO concentrates are compared with UF ones at pH 6.50. Lowering the pH of RO concentrates from 6.50 to 5.60 led to a significant ($P < 0.05$) decrease in the calcium mineralisation of casein micelles for each concentration. Several studies have already demonstrated

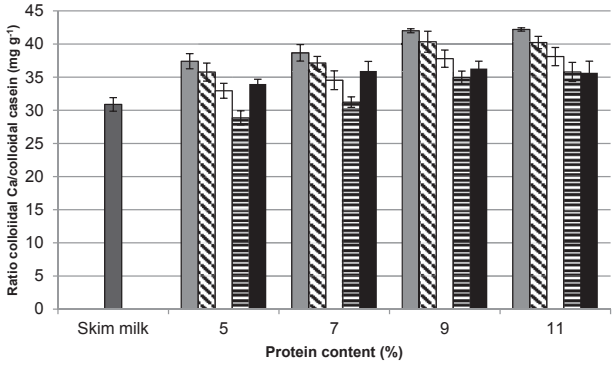

Fig. 1. Impact of pH on colloidal calcium/colloidal casein ratio of RO-concentrated skim milk (■, pH 6.50; ▨, pH 6.20; □, pH 5.90; ▤, pH 5.60) at 5%, 7%, 9% and 11% protein content in comparison with UF concentrates (■, pH 6.50). Error bars correspond to t standard deviation (n = 3).

that the dissociation of salts from casein (calcium, magnesium, inorganic phosphate and citrate) is increased with a decrease in pH (Dalgleish & Law, 1989; Le Graët, 1999). The results showed that the solubilisation pattern involving these salts was dependent on the milk protein content. As previously reported by Le Graët (1999), a lower pH is needed to achieve the same level of solubilisation when increasing milk protein concentration. For each RO concentration, the pH needed to reach the same colloidal calcium/colloidal casein ratio as the UF concentrates has been calculated (5%, pH 6.05; 7%, pH 6.00; 9%, pH 5.75; 11%, pH 5.60) from Fig. 1 and have been used in the production of model cheeses. However, for the cheesemaking experiments, the renneting pH of all concentrates was 0.3 pH units lower than the pH used for salt partitioning experiment. As explained in Section 2.6, this change in pH was made to simulate the demineralisation traditionally obtained by the use of acid lactic bacteria during cheesemaking.

3.3. Rennet coagulation kinetics as a function of the concentration

The rennet coagulation kinetics regarding RO and UF concentrates were analysed by dynamic rheology. Three parameters have been extracted from the data: the rennet coagulation time (RCT), the maximal firming rate of the gel (MFR) and the storage modulus of the gel after 60 min (G′ at 60 min). The RCT of UF and RO concentrates (pH 6.50) were monitored at five concentrations (skim milk, 5%, 7%, 9% and 11% protein content) and are presented in Fig. 2a. The RCT of UF concentrates was not significantly ($P > 0.05$) affected by the increase of protein concentration and remained similar to the one obtained for skim milk. Our results are in accordance with several studies (Lauzin et al., 2018; Mistry & Maubois, 2017; Sandra et al., 2011; Sharma, Hill, & Mittal, 1993). However, Waungana et al. (1998) reported no effect of concentration by UF on RCT at natural pH, but a decrease of it when the concentrates were adjusted to pH 6.50. The literature discrepancy about the effect of protein concentration on RCT in UF concentrates can be attributed to the different methods employed, the definition of coagulation time, the processing history of concentrated milk (Sandra et al., 2011) or the rennet concentration, defined in terms of milk volume or casein weight.

Fig. 2a also shows a significantly ($P < 0.05$) higher RCT for RO concentrates than for UF ones, for each of the concentration studied. Increasing RO concentration resulted in a sharp proportional increase of RCT, from 16.32 min (skim milk) to 39.97 min (RO, 11%). The RCT is the sum of the enzymatic hydrolysis phase and the aggregation phase leading to an increase of G′ by 1 Pa. Bienvenue, Jiménez-Flores, and Singh (2003) reported that κ-casein hairy layers may be affected by shielding of charge if additional colloidal calcium phosphate is deposited on the surface of casein micelles. This could result in an extended hydrolysis phase. The follow-up of caseinomacropeptide release after renneting was studied on RO and UF concentrates at pH 6.50 by Lauzin, Bérubé, Britten, and Pouliot (2019). The authors found no significant ($P > 0.05$) difference between the kinetics of κ-casein in skim milk, RO and UF concentrates, indicating that the enzymatic hydrolysis phase was not responsible for variations in RCT. The apparent viscosity was shown to be higher for RO concentrates than for skim milk and UF concentrates (Bienvenue et al., 2003; Lauzin et al., 2018). This higher viscosity, caused by higher concentrations of lactose and salts, can slow down the diffusion of renneted casein and contribute to increasing RCT.

The MFR of UF and RO concentrates was also monitored as a function of the protein concentration, as shown in Fig. 2b. In UF concentrates, an increase in the protein content led to a higher MFR of the gel. As the protein content increased, the volume fraction of casein micelles also increased and the distance between them

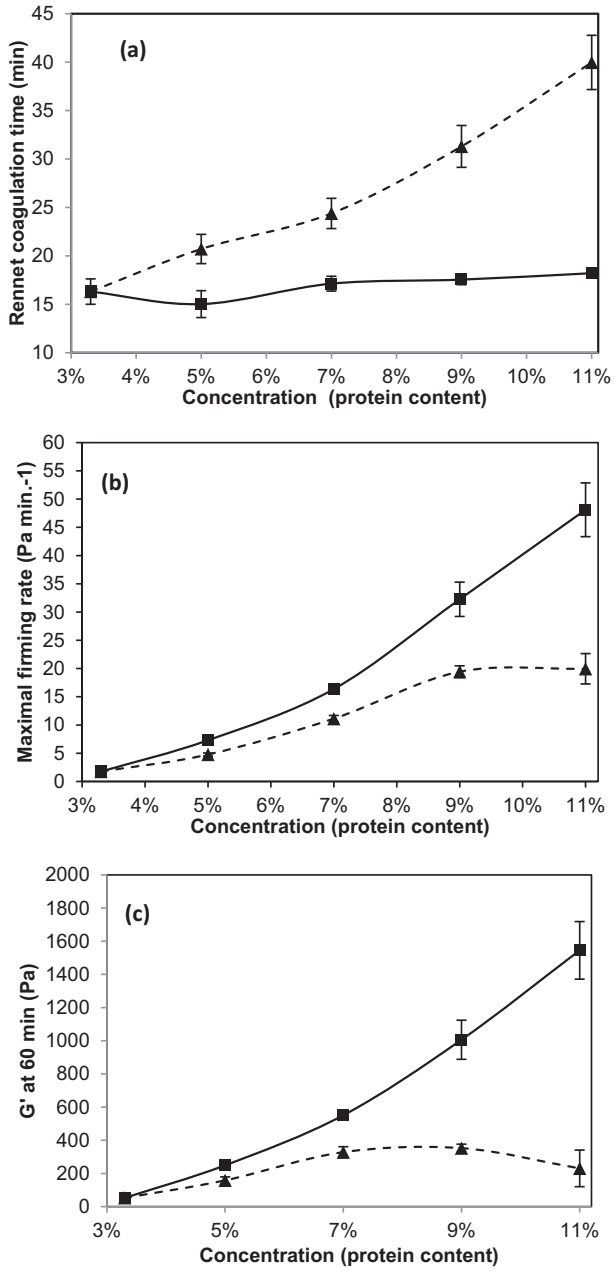

Fig. 2. Effect of protein concentration, for UF (■) and RO (▲) concentrates, on (a) the rennet coagulation time; (b) the maximal firming rate; and (c) the storage modulus at 60 min. Error bars correspond to standard deviation (n = 3).

became smaller. This is likely to increase the frequency of collisions, the number of bonds and the contact area in the gel network, causing a higher firming rate (Sandra et al., 2011). In RO concentration, the increase of MFR was much slower than in case of UF concentration. Also, at a protein content of 9%, the MFR reached a plateau. This effect could be explained by the increase in the viscosity of concentrates, which can slow down the movement of casein micelles (Guinee et al., 1997; Karlsson, Ipsen, & Ardö, 2007b), or by the increase of casein mineralisation. Malacarne et al. (2014) reported that an excessive mineralisation of casein micelles could reduce the number of phosphate groups available for curd formation during the aggregation phase of rennet coagulation. An increase in casein mineralisation also leads to a decrease of Ca^{+2} in the serum phase, which can be detrimental for cross-linking rennet-altered micelles (Fox, Guinee, Cogan, & McSweeney, 2017).

The storage modulus (G′) of the gel was recorded 60 min after rennet addition and the results are presented in Fig. 2c. Using UF concentration, the increase of gel firmness was proportional to the protein content in concentrates, with values varying from 59.64 Pa (skim milk) to 1545.13 Pa (UF, 11%). These results are in full agreement with other studies (Sandra et al., 2011; Waungana et al., 1998) and are attributed to the closer proximity and the higher concentration of casein micelles. As for RO concentrates, the firmness of the gel at 60 min has increased from 52.64 Pa (skim milk) to 352.55 Pa (RO, 9%) and then begun to decrease at a protein content of 9%. This was the net result of an extended rennet coagulation time and the reach of a plateau in the maximal firming rate of the gel.

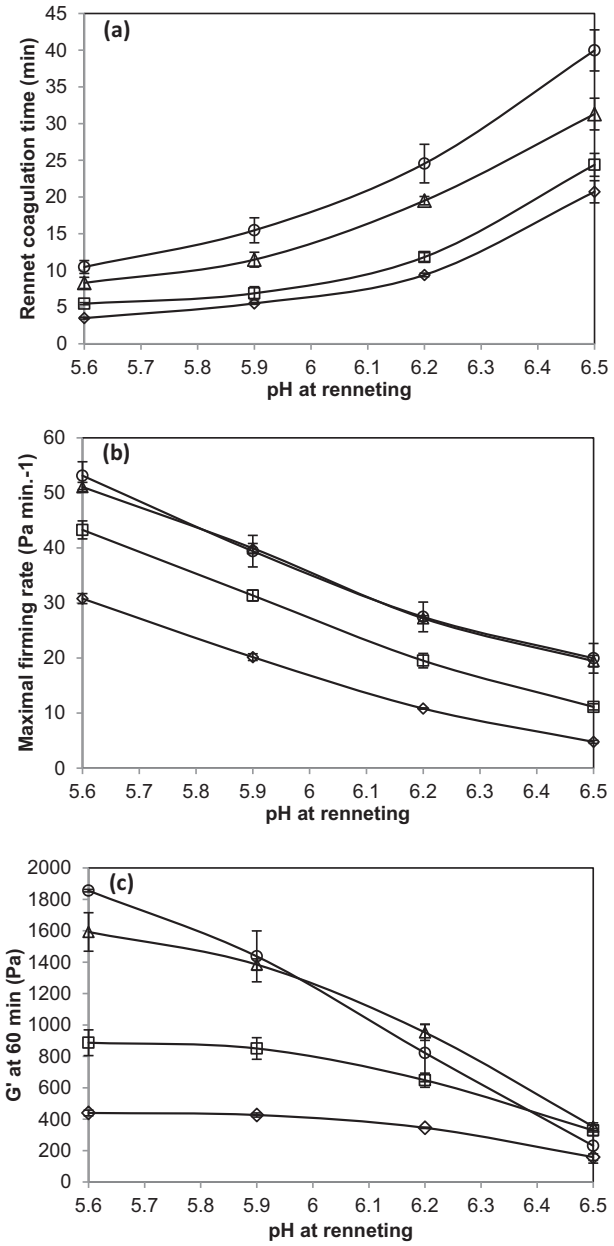

Fig. 3. Effect of renneting pH and RO concentration (◇, 5%; □, 7%; △, 9%; ○, 11%) on: (a) the rennet coagulation time; (b) the maximal firming rate; and (c) the storage modulus at 60 min. Error bars correspond to standard deviation (n = 3).

3.4. Rennet coagulation of acidified RO concentrates

The rennet coagulation kinetics regarding RO concentrates have been characterised as a function of the renneting pH (6.50–5.60). Fig. 3a shows the impact of the latter on the RCT of RO concentrates. As previously observed by Lucey (2002), lowering the renneting pH led to a significant ($P < 0.05$) reduction in terms of RCT, whatever the protein content. For RO concentrates containing 11% of proteins, it decreased from 39.97 min (pH 6.50) to 10.46 min (pH 5.60). Lowering renneting pH increases the rennet activity, which accelerates the κ-casein hydrolysis. A low pH also reduces the electrostatic repulsion between casein micelles, increasing the aggregation rate of paracasein (Karlsson et al., 2007a). It has been shown that, at lower pH values, the micelle aggregation phase starts at a lower degree of κ-casein hydrolysis (Lauzin et al., 2019).

The results, presented in Fig. 3b, show that a decrease in pH from 6.50 to 5.60 led the MFR to increase from 19.95 Pa min^{-1} to 53.13 Pa min^{-1} for RO with a 11% protein content. The increase in the MFR associated with a decrease in pH was similar for all the concentration studied. It is known that acidification decreases the net negative charge on casein micelles, leading to a reduced electrostatic repulsion between them and to an increase in the aggregation of casein (Karlsson et al., 2007a; Mishra, Govindasamy Lucey, & Lucey, 2005). Lowering the pH promotes the colloidal calcium phosphate solubilisation, which leads to an increase of Ca^{2+} activity. This ion plays an important role in reducing repulsion between negatively charged caseins and leads to a faster rate of micelle aggregation and bond formation (Dalgleish, 1983; De La Fuente, 1998; López, Lomholt, & Qvist, 1998). Increasing the MFR of RO concentrates by lowering the renneting pH could be an important step to improve their cheesemaking properties.

The storage modulus values at 60 min regarding pH-adjusted RO concentrates are presented in Fig. 3c. The acidification of RO concentrates resulted in an increase of G′ at 60 min because of a lower RCT and a higher MFR compared with concentrates at pH 6.50. The increase in G′ at 60 min associated with the lowering of the pH between 6.50 and 5.60 was more pronounced for RO with a protein content of 11% (from 231 Pa to 1855 Pa) than for the one with 5% (from 158 Pa to 440 Pa).

3.5. Model cheese production

Model cheeses have been designed to study the effect of concentration and acidification on paracasein gel matrix properties. Moisture, protein content and composition of protein, lactose, ash and calcium on a dry matter basis are presented in Table 2. The moisture of the control skim milk cheese, with 56.15%, was similar to other values found in literature (Lauzin et al., 2018). Cheeses produced from UF concentrates tended to be high in moisture compared to controls made from skim milk. However, the moisture content of cheese did not significantly ($P > 0.05$) change as the protein content of the UF concentrates increased. These results disagree with those of Guinee et al. (2006), who reported that the moisture content of UF cheese decreased with increasing milk protein concentration. In our study, all the gels were cut at a constant firmness of 40 Pa, so the cutting step cannot be responsible for this discrepancy. However, with an increase in concentration, it was observed that the curd particles tend to agglomerate in the vat during the cooking phase of the cheese production. The collisions between them were then reduced, which can account for a decrease in syneresis from the gel (Lucey et al., 2003). The moisture regarding cheeses made from RO milk at pH 6.20 was higher than the one obtained from UF milk at the same pH value, which can be attributed to a lower protein rearrangement of the gel network. Our results showed that lowering the pH of RO concentrates reduced the cheese moisture since all resulting acidified cheeses had a significantly lower ($P < 0.05$) moisture content than those at pH 6.20. A faster curd formation at lower pH promotes whey expulsion (Daviau, Famelart, Pierre, Goudédranche, & Maubois, 2000), leading to a drier cheese.

In general, increasing milk protein levels in the range of 5–11% by ultrafiltration had little effect on cheese composition. Protein, lactose and ash contents did not significantly ($P > 0.05$) change with the increased concentration. The same finding was observed by Guinee et al. (1994) for cheeses made from milk concentrated by UF (3–8.2% protein content). The dry matter protein content in cheeses made from RO milk at pH 6.20 decreased as the concentration increased, but the lactose content increased significantly ($P < 0.05$), as reported by Agbevavi et al. (1983) and Bynum and Barbano (1985). However, the high lactose concentration in cheese may limit the application of RO in cheese technology, as it can lead to impaired organoleptic qualities due to increased lactic acid fermentation during ripening (Hydamaka et al., 2000). The acidification of RO concentrates promoted a decrease regarding the lactose content, as a result of lower moisture degree in cheese. As expected, the acidification did the same for both the ash content and the calcium/protein ratio because of the colloidal calcium phosphate solubilisation. The values were similar to those obtained from skim milk and control cheeses made from UF milk.

Cheese yield, moisture-adjusted yield and true protein retention of model cheeses are presented in Table 3. As expected, increasing the protein content in both UF and RO concentrates led to an increase in cheese yield. For the same renneting pH (6.20), yields were significantly ($P < 0.05$) greater for RO than for UF cheeses,

Table 2
Cheese composition as affected by concentration, renneting pH and type of concentrate.[a]

Milk protein level (%)		Renneting pH	Cheese moisture (%)	Protein (%)	Protein (% dry matter)	Lactose (% dry matter)	Ash (% dry matter)	Calcium (% dry matter)	Calcium (mg g^{-1} protein)
Skim milk		6.20	56.15 ± 1.48^{bcd}	38.51 ± 1.49^{fgh}	87.82 ± 0.79^{i}	4.85 ± 0.17^{a}	6.75 ± 1.09^{a}	2.24 ± 0.31^{def}	25.54 ± 3.76^{abc}
5	RO	6.20	58.67 ± 1.30^{ef}	33.94 ± 1.69^{d}	82.09 ± 1.54^{g}	8.54 ± 0.92^{c}	8.40 ± 0.42^{de}	2.31 ± 0.10^{ef}	28.15 ± 1.74^{bcd}
	RO	5.75	53.17 ± 1.60^{a}	39.37 ± 1.61^{h}	84.07 ± 0.58^{gh}	6.72 ± 1.57^{abc}	6.88 ± 0.29^{ab}	2.04 ± 0.06^{bdc}	24.25 ± 0.58^{a}
	UF	6.20	57.65 ± 0.84^{cdef}	36.63 ± 0.83^{ef}	86.51 ± 0.26^{hi}	5.45 ± 0.69^{a}	7.70 ± 0.28^{bcd}	2.13 ± 0.12^{cde}	24.61 ± 1.49^{a}
7	RO	6.20	59.26 ± 1.04^{f}	30.61 ± 1.30^{c}	75.11 ± 1.58^{e}	15.15 ± 1.99^{e}	8.45 ± 0.24^{de}	2.02 ± 0.06^{bcd}	26.86 ± 0.29^{abc}
	RO	5.70	54.97 ± 2.61^{b}	35.33 ± 3.22^{de}	78.36 ± 2.63^{f}	10.66 ± 2.05^{d}	7.63 ± 0.12^{bcd}	1.97 ± 0.06^{bc}	25.11 ± 0.29^{ab}
	UF	6.20	58.82 ± 0.62^{ef}	35.37 ± 0.58^{de}	85.89 ± 0.12^{hi}	6.29 ± 0.11^{a}	8.18 ± 0.23^{cd}	2.34 ± 0.04^{ef}	27.19 ± 0.53^{bc}
9	RO	6.20	61.57 ± 0.90^{g}	25.34 ± 2.45^{b}	65.85 ± 4.78^{c}	22.58 ± 0.95^{g}	8.35 ± 0.45^{de}	1.94 ± 0.06^{abc}	29.53 ± 1.74^{d}
	RO	5.45	56.47 ± 0.34^{bcd}	30.76 ± 0.31^{c}	70.67 ± 1.00^{d}	17.54 ± 0.24^{f}	7.40 ± 0.20^{abc}	1.87 ± 0.11^{abc}	26.41 ± 1.23^{bcd}
	UF	6.20	58.87 ± 1.01^{ef}	35.15 ± 0.91^{de}	85.46 ± 0.13^{hi}	5.57 ± 1.78^{ab}	8.06 ± 0.22^{cd}	2.45 ± 0.04^{f}	28.65 ± 0.41^{cd}
11	RO	6.20	62.36 ± 0.74^{g}	20.33 ± 1.05^{a}	54.00 ± 2.20^{a}	33.26 ± 0.27^{i}	9.17 ± 0.76^{e}	1.86 ± 0.15^{ab}	34.49 ± 4.04^{e}
	RO	5.30	57.16 ± 1.89^{cde}	26.22 ± 2.74^{b}	62.77 ± 1.89^{b}	25.42 ± 1.54^{h}	7.72 ± 0.18^{cd}	1.70 ± 0.01^{a}	27.08 ± 1.03^{cd}
	UF	6.20	57.80 ± 0.50^{def}	36.82 ± 0.25^{efg}	86.27 ± 1.10^{i}	7.49 ± 0.30^{bc}	8.27 ± 0.00^{d}	2.32 ± 0.08^{ef}	26.61 ± 0.65^{bcd}

[a] Data are based on triplicate experiments; values in the same column without a common superscript letter are significantly different (Tukey, $P < 0.05$).

Table 3
Mean yield, moisture adjusted yield and protein retention of model cheeses as influenced by concentration, renneting pH and type of concentrate.[a]

Milk protein level (%)		Renneting pH	Yield (%)	Moisture adjusted yield (%)	True protein retention (%)
Skim milk		6.20	7.47 ± 0.75^{a}	8.33 ± 1.09^{a}	79.26 ± 1.01^{cd}
5	RO	6.20	12.86 ± 0.44^{c}	13.41 ± 0.58^{b}	79.52 ± 0.11^{d}
	RO	5.75	10.22 ± 0.51^{b}	12.52 ± 0.35^{b}	79.56 ± 0.46^{d}
	UF	6.20	11.08 ± 0.20^{b}	11.86 ± 0.09^{b}	79.79 ± 0.33^{d}
7	RO	6.20	20.75 ± 0.74^{e}	21.33 ± 0.76^{e}	78.21 ± 0.32^{abc}
	RO	5.70	15.91 ± 1.27^{d}	18.85 ± 0.01^{d}	79.32 ± 0.43^{cd}
	UF	6.20	16.04 ± 0.12^{d}	16.62 ± 0.20^{c}	79.36 ± 0.49^{d}
9	RO	6.20	32.57 ± 1.65^{h}	30.13 ± 0.78^{g}	77.51 ± 0.35^{a}
	RO	5.45	23.54 ± 0.29^{f}	27.36 ± 0.80^{f}	77.95 ± 0.82^{ab}
	UF	6.20	20.92 ± 0.44^{e}	21.66 ± 0.28^{e}	78.93 ± 0.39^{bcd}
11	RO	6.20	46.60 ± 1.76^{j}	40.16 ± 3.25^{h}	77.34 ± 0.54^{a}
	RO	5.30	36.38 ± 0.76^{i}	42.39 ± 3.60^{h}	78.81 ± 0.74^{ab}
	UF	6.20	26.34 ± 0.48^{g}	28.15 ± 0.11^{fg}	77.84 ± 1.24^{a}

[a] Data are based on triplicate experiments; values in the same column without a common superscript letter are significantly different (Tukey, $P < 0.05$). Moisture adjusted yield was adjusted to 60% moisture content; true protein = TN−NPN.

which is in agreement with Lauzin et al. (2018). Higher values for RO concentrates are partly explained by the moisture content (Table 2). However, moisture-adjusted yields showed a similar trend, confirming the significant contribution of lactose and minerals to the cheese yield. The acidification of RO concentrates significantly reduced yields ($P < 0.05$), but had no significant effect on true protein retention ($P > 0.05$). Indeed, the lower moisture content in cheeses made from acidified RO concentrates (Table 2) is mainly responsible for lower yields. However, moisture-adjusted yields were also lower in these cheeses made from acidified concentrates, which confirms that reducing the pH of RO concentrates promotes the solubilisation of colloidal salts and reduces ash retention. A lower yield is detrimental from a financial viewpoint, but the yield of cheeses made from acidified RO concentrates still remained similar to or higher than that from UF concentrates.

3.6. Cheese rheology

The viscoelastic properties of cheese were determined by dynamic low amplitude oscillatory rheology. The effect of concentration and renneting pH on the complex modulus (G*) of cheese measured at 1 Hz is presented in Fig. 4. This parameter is an indicator of the number and the strength of the bonds forming the paracasein matrix (Banville et al., 2014). The G* of cheeses made from RO milk at pH 6.20 was lower than that of acidified ones. As shown in Table 2, the protein content in the RO acidified cheese was higher, which means that there were more bonds in the matrix.

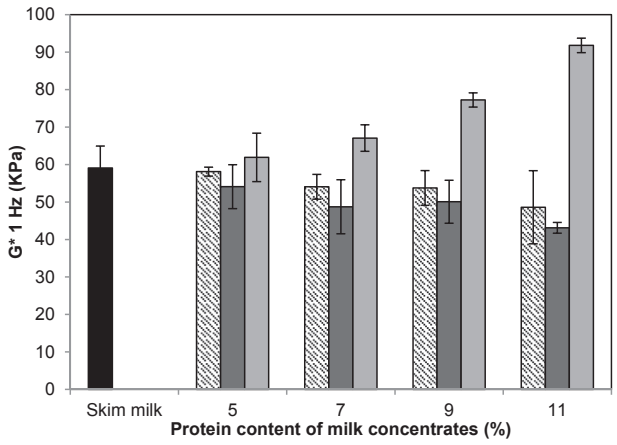

Fig. 4. Effect of concentration and renneting pH on the complex modulus of cheese measured at 1 Hz (G^*_1) (■, skim milk; □, UF pH 6.0; ■, RO pH 6.20; ■, RO low pH). Error bars correspond to standard deviation (n = 3).

These cheeses also had lower moisture content. As reported in Perreault et al. (2017), the complex modulus is highly dependent on the moisture of cheeses and tends to increase with a decreasing moisture content. By comparing G* of cheeses made from RO milk with those made from UF milk at pH 6.20, they were not significantly different ($P > 0.05$).

However, as presented in Table 2, the protein content in cheeses made from RO milk at pH 6.20 was lower than that in UF ones. Consequently, a higher G* was expected for the UF cheeses. It was hypothesised that the cheese aqueous phase, which is loaded in lactose for RO cheeses and the higher protein mineralisation may increase the complex modulus of these cheeses. Fig. 4 also shows a significant raise ($P < 0.05$) of G* associated with the increase in protein levels of concentrates for RO acidified cheeses. However, as presented in Table 2, the moisture content of these cheeses increased and their protein content decreased as the protein level of concentrates increased, both factors generally decreasing the G* value.

The frequency dependence of the complex modulus G^*_{fd} is a measure of how fast the bonds interacting in the cheese matrix can reorganise or recover upon deformation. Slowly recovering bonds would result in a higher frequency dependence (Banville et al., 2014). There was no significant ($P > 0.05$) impact of milk concentration on the frequency dependence of complex modulus (data not shown). The values of tan δ_{fd}, varied from 0.034 to 0.052, but was not significantly ($P > 0.05$) different between the treatments (data not shown). Since the loss tangent (tanδ) corresponds to the viscous to elastic moduli (G″/G′) ratio (Lucey et al., 2003), this suggests that both components varied in the same way with increased frequencies (Banville et al., 2014; Perreault et al., 2017).

4. Conclusion

The aim of this study was to investigate the effect of a pH adjustment regarding RO concentrates (5%–11.0% protein content) on the salt partitioning, the rennet coagulation kinetics and the cheesemaking properties. Increasing RO concentration has negatively affected the rennet coagulation kinetics in comparison with skim milk and UF controls. An increase of the casein mineralisation was also observed. Lowering the pH was an important factor to improve the rennet coagulation properties, as it led to a decrease of rennet coagulation time a faster maximal firming rate, as well as a promoted solubilisation of colloidal salts.

The experiments on model cheeses showed altered properties in RO concentrates, featuring higher moisture and lactose contents as well as calcium/protein ratio. The moisture-adjusted yield in cheese made from RO milk was higher in comparison with those

made from UF and skim milk. The acidification of RO concentrates lowered the moisture, salt and lactose contents, yet still having this higher moisture-adjusted yield than that of control cheeses made from UF milk. The complex modulus of these cheeses (RO acidified) was also higher because of reduced curd moisture and higher protein content. These results suggest that acidification is an essential step for improving the cheesemaking with RO concentrates. The high lactose content is interesting as it increases the cheese yield, but may be a problem due to the post-acidification by lactic acid fermentation during the ripening (Hydamaka et al., 2000), particularly with cheese made from highly concentrated milk.

Acknowledgements

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), Novalait Inc. and Fonds de recherche du Québec—Nature et technologies (FRQNT). The authors thank Véronique Richard, Diane Gagnon, Mélanie Martineau and Pascal Lavoie from the Department of Food Science at Laval University for their technical assistance during the experiments and analysis and Dominique Fournier for the editing of this manuscript.

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