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FRESH CHEESE FROM CASEIN CONCENTRATES

Manufacture and characterization of acid coagulated fresh cheese made from casein concentrates obtained by acid diafiltration

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INTERPRETIVE SUMMARY

Fresh cheese manufacture using microfiltration

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Using concentrated milk with increased protein content is growing in the fresh cheese industry for its economic sustainability. However, an increased protein concentration in milk is associated with an increased colloidal mineral content. This relative excess in minerals causes textural and flavor

defects in the produced cheese. The objective of this study was to produce fresh cheese from high protein milk which pH and mineral content were adjusted using a slight acid diafiltration process. Different types of acidifying agents were used during processing and their influence on the fresh cheese composition, acidification kinetics, texture and sensory properties were assessed.

ABSTRACT

This study aimed to investigate the production of acid coagulated fresh cheese by using slightly acid diafiltered (DF) microfiltered (MF) casein concentrates (8% protein). Three different acidifying agents were tested during DF; carbon dioxide, lactic- and citric acid. Fresh cheese was manufactured using acid-DF casein concentrates, or casein concentrates DF with just water and compared to cheese manufactured MF casein concentrates without DF. The fresh cheeses were characterized for composition, rheological- and sensorial properties. Acid-DF casein concentrates improved the acidification kinetics during cheesemaking and reduced casein leakage to the cheese whey as compared with cheese from regular MF casein concentrate. Among the rheological properties investigated in this study, the storage modulus of the fresh cheese was higher when DF of the casein concentrate was made with non-acidified DF-water or when DF-water was acidified with citric. However, fresh cheese made from casein concentrate diafiltered with DF-water acidified by citric acid was most liked in a sensory ranking test.

Keywords: microfiltration, acid diafiltration, casein concentrates, fresh cheese, rheology

INTRODUCTION

The mineral content of milk and its acidification kinetics during cheesemaking, play a major role in the rheological properties of cheese (Maubois and Kosikowski, 1978, Kindstedt and Guo, 1998). The hydration of the protein matrix in cheese and its textural aspect are controlled by the total calcium content and its repartition between the soluble and colloidal phase (Shehata et al., 1966, Guinee et al., 2002, McMahon et al., 2005). The concentration of milk proteins by means of microfiltration (MF), as a pre-preparation step for cheesemaking, influences the chemical composition of the cheese milk (Marella et al., 2013). As casein concentration is increased, the mineral content and buffering capacity are also increased (Salaün et al., 2005). This modifies cheese making parameters such as the performance of the starter culture with respect to the acidification kinetics and its ability to reach the final pH. During production of fresh cheese, the fermentation of skim milk at ~30 °C to pH ~4.6 causes solubilization of calcium. The rate of change in pH during fermentation is controlled by the buffering capacity of the milk (Salaün et al., 2005). The microfiltered casein concentrate with its increased buffering capacity requires more acid production during fermentation, to reach the same pH and calcium solubilization as in skim milk (St-Galais et al., 1992). Finally, the rheological properties and flavour of acid coagulated fresh cheese made from casein concentrates obtain textural- and flavour defects such as too viscous/weak texture and bitter flavour (de la Fuente, 1998, Guinee et al., 2002, Heino et al., 2010). There are 3 different processing options to manufacture fresh cheese: 1) fermentation of milk and concentration of the coagulum, 2) concentration of milk and fermentation of the concentrate and 3) a hybrid processing of 2) and 1) (concentration of milk, fermentation of the concentrate, concentration of the coagulum). Concentration of milk by microfiltration (MF) offers a flexible

option for fresh cheese producers who wants to maximize recovery of whey protein (WP) in a native state for other applications (Saboya and Maubois, 2000, Marella et al., 2013).

Semi-hard and hard cheese manufactured using MF casein concentrates (MF CC) had similar texture defects as cheese produced from UF concentrates due to high buffering capacity and calcium concentration (Soodam and Guinee, 2018)(Neocleous et al., 2002b, Neocleous et al., 2002a, Heino et al., 2010, Outinen et al., 2010). Limited research is, however, available on the impact of using MF CC for acid coagulated fresh cheese manufacture and quality (Farkye, 2017). Korolczuk and Mahaut (1991) compared structure of fresh cheese obtained by MF (above mentioned process #2) and conventional production (above mentioned process #1). The authors reported that addition of insufficiently denatured whey protein to the cheese milk prior to fermentation lead to weak structure due to the WP loss in the whey. Imai et al. (2010) patented a method for fresh cheese production from MF concentrates by using acetic acid for cold acidification of the casein concentrates followed by heat treatment to form the curd. The claimed advantage was to produce fresh cheese with a low WP content, of which they held responsible for flavor and texture defects. Pre-acidification, addition of calcium chelators or diafiltration (DF) can be used to reduce the buffering capacity of the MF concentrates (St-Galais et al., 1992, Law and Leaver, 1998, Hurt and Barbano, 2010). A theoretical design of a combined MF-DF that uses acidified water for DF in a 2-stage filtration, was proposed by Nelson and Barbano (2005) to enable reduction of minerals in the retentate for Cottage Cheese production. But, to the authors knowledge, no published studies assessed the method. Organic acids like lactic- and citric acid are commonly used in the cheese industry for pre-acidification of cheese milk to reduce manufacturing time (McSweeney et al., 2017), or to produce acid/heat-coagulated type cheeses like Ricotta,

Quark and Mascarpone where rennet may or may not be added. Citric acid may be used to pre-acidify cheese milk in Mozzarella production, where it contributes to curd softness and increased meltability (Zisu and Shah, 2007, Francolino et al., 2010). However, higher concentrations of citric acid lead to a harder product (Arora and Khetra, 2017). Recently, Schäfer et al. (2019) published a method for fresh cheese manufacture using MF casein concentrates with reduced Ca to casein content. The skim milk was cold acidified to pH 6.2 by citric acid (1M, dropwise addition under continuous stirring) prior to MF, then the CC was further cold acidified the same way with the citric acid to pH 5.6, followed by six-repeated DF with demineralized water to remove Ca. The cheese obtained a reduced bitter taste and a firmer gel as compared with cheese from non-acidified MF CC. This process is time consuming and complex to up-scale since many process steps are included.

As implementation of a MF process in fresh cheese production require optimization, direct acidification of milk to reduce pH prior to the MF process does not present an attractive solution for dairy ingredient manufacturers. Acidification of milk to pH 6.0 and below, to reduce the colloidal Ca- and Mg phosphate salts (Sharma and Reuter, 1993), results in an acid permeate of reduced value for WP production. The pH affects the WP functionality such as reduced gelation temperature, increased water absorption and viscosity. A previous study, (Gaber et al., 2020a) showed that a pH reduction of the MF CC by 0.1 unit by acid-DF, modified the CC composition. The Ca^{2+} solubilization increased and the WP content was reduced in acid-DF CC. Lactic acid DF increased Ca^{2+} solubilization, while carbonation reduced the P_i and WP content of the MF CC. Therefore, the mineral composition and storage modulus of an acid coagulated fresh cheese would consequently be expected to be modified.

The aim of this study was to investigate the effect of acidified MF casein concentrate by 0.1 unit pH reduction on the production of acid coagulated fresh cheese in a hybrid process as mentioned above in process #3. The casein concentrate was diafiltered using water or acidified water (acidified with citric acid, lactic acid or carbonation). Fresh cheese was produced using the MF DF CCs or MF CC (control). The effect of the different treatments was compared regarding fermentation during cheese production, as well as the composition, rheological and sensory properties of the cheese.

MATERIALS AND METHODS

Casein concentrates preparation

Flow charts of the production process with MF, DF and cheese making for the reference and experimental cheeses are shown in Figure 1. The experiment consisted of 4 replicate blocks; within each replicate block, a batch of raw milk (1800 L each) were skimmed (Westfalia Separator AG, MSD50-01-076, Oelde, Germany) at 55 °C and pasteurized at 73 °C for 15 s using a plate heat exchanger (A3-HRB, Alfa Laval, Lund, Sweden). The batch of pasteurized skim milk was further split into two equal sub-batch quantities (900 L). Each sub-batch was microfiltered (MF) (UF/MF pilot MCC RV 01118340, APV, Silkeborg, Denmark) using a 0.14-µm ceramic membrane (INSIDE CéRAM™, TAMI Industries, GEA, Nyons, France) at 50 ± 0.1 °C and uniform trans-membrane pressure (UTMP) at 51 ± 4 kPa, to a volume concentration factor (VCF) of ~2.5. Approximately 250 L of 8% ± 0.1 (w/w) casein concentrate (CC) was produced per sub-batch. The macro composition of the CC during MF was determined by a MilkoScan FT1 (CombiFoss 6500, Hillerød, Denmark) using Fourier transform infrared analysis (FTIR). From each MF CC sub-batch, 20 L were taken for the reference fresh cheese manufacture (MFR) and the rest was divided

in two batches of 100 L, which underwent a specific DF treatment (as described under). Two DF runs were performed per sub-batch each production day. The DF treatment was done by adding 30 L DF-water to the 100 L of MF-retentate, reaching a diavolume of (DV) 0.3. The DF-water used was either pure water (pasteurized tap water with an average °dH of 2.9) or acidified water. The MF-DF process continued until regaining 8% ± 0.1 (w/w) protein concentration in the CC. The individual acidifying agents used for acidification of the DF-water were; (1) 10 mM lactic acid (DL-lactic acid, 85% w/w, Sigma-Aldrich, Missouri, USA) (DF-water final pH of 3.1), (2) 10 mM citric acid (10% w/w citric acid monohydrate solution, citric acid monohydrate ACS reagent, ≥ 99.0%, Sigma, Germany) (DF-water final pH of 3.0), and (3) Carbonation by using a liquid CO_2 tank (approx. 1.69 gL^{-1} CO_2) (BIOGON® C, AGA, Oslo, Norway) (final pH of DF media: 4.6). The selected concentration of each acidifying agent was previously adjusted to correspond to the amount required to obtain a drop in pH of the casein concentrate by 0.1 unit after addition of acidified water (Gaber et al., 2020a). The 4 different DF treatments were randomized within each replicate block. Table 1 shows the abbreviations used to describe products issued from each of the experimental treatments.

Acid coagulated fresh cheese manufacture

The MF-DF experimental acid coagulated fresh cheese made were: 1) fresh cheese made from MF-DF-CC using pasteurized water for DF (RDR); 2) fresh cheese made from MF-DF-CC using pasteurized water acidified with lactic acid for DF (LDR); 3) fresh cheese made from MF-DF-CC using pasteurized water acidified with citric acid for DF (CDR); and 4) fresh cheese made from MF-DF-CC using carbonated pasteurized water for DF (ODR). The reference fresh cheese made from MF CC (MFR).

A second pasteurisation was performed for the MF CC and MF-DF CC at 73 °C for 15 s, then cooled down to 30 °C and 5 L were transferred into 10 L cheese vats. Starter culture Probat 505 (Probat 505 FRO 500 DCU, CHOOZIT™ Cheese Cultures, Danisco) was mixed and split into several 200 mL sterile flask and stored at -40 °C until usage. The vat was inoculated with 2 % (v/v) starter culture, by first pipetting 1 mL from the thawed flask into 50 mL of CC, mixed and the entire volume was added to the cheese vat at 30 °C. The starter culture was added to the cheese vat under continuous stirring for 10 min to ensure uniform mixing, and the cheese vat was further incubated for 17 to 18 h until pH 4.7. Continuous pH measurements were performed during fermentation using a 742020 sensIONTM+pH31 meter with 5011T probe (Hach Lange GMBH, Dusseldorf, Germany) connected to a LabCom V2.1 software (Hach, Lange GMBH, Germany). At pH 4.7 the curd was cut and separated using a hanging cloth and left for draining at 4- 5 °C for 4 h. The mass of the drained curd was weighted, and samples of cheese and whey was collected for analysis. The cheese was further mixed using a colloid mill (Fryma Maschinen AG CH-4310 Rheinfelden, Switzerland). The curd was packed in ~200 and 500 g plastic cups with lids and stored at 4- 5 °C until further analysis.

Compositional Analysis

Total nitrogen (TN) of all samples were determined using the Kjeldahl method (IDF, 2001, 2004). The results were multiplied by the factor 6.38 to calculate the percentage of total protein. Identification of the protein composition in the cheese whey were performed using capillary electrophoresis (CE) as described by Jørgensen et al. (2016). The total content of calcium (Ca), phosphorous (P), potassium (K), sodium (Na) and magnesium (Mg) were quantified by inductively

coupled plasma mass spectrometry (ICP-MS) as described by Jørgensen et al. (2015). Inorganic phosphate (P_i) was analysed using an Agilent (G1600AX) Capillary electrophoresis (CE) with Agilent ChemStation software (Agilent technologies, Waldbronn, Germany), according to Izco et al. (2003) and with modifications as described by Gaber et al. (2020b). Calcium ion activity (Ca^{2+}) was determined using an Orion 97-20 calcium ion selective electrode (Calcium ionplusR Sure-FlowR Plastic Membrane Combination ISE, Thermo Scientific, Chelmsford, USA) with an mV meter (PHM290, pH-STAT Controller, MeterLabTM, Radiometer Analytica, Copenhagen) according to the manufacturer's instructions. Serial dilutions of a calcium standard solution were prepared and measured before and after the samples. A calcium ionic strength adjuster (ISA) (Ca.No. 932011, Thermo Scientific, Chelmsford, USA) was added to the standards and the samples, mixed and incubated at 30 °C for 30 min prior to measurement. Analysis of individual samples were run in duplicates. Organic acids (lactic and citric acid), and carbohydrates (lactose, galactose, and glucose) were quantified by High Performance Liquid Chromatography (HPLC) as described by Moe et al. (2013). The individual samples were analysed in triplicate unless otherwise stated.

Cheese yield was calculated according to equation (1) (Banks, 2007):

$$Yield = \frac{m_{cheese}}{m_{CC}} \times 100 \qquad (1)$$

Where m_{cheese} is the weight of the cheese and m_{CC} is the weight of the initial casein concentrates.

Rheological measurements

Small amplitude oscillatory shear experiments (SAOS) of fresh cheeses were conducted after 7 days of storage using a MCR 301 Rheometer with a plate-plate (PP50 diameter: 50mm) measuring

geometry (Anton Paar GmbH, Graz, Austria). A strain sweep test was performed with a logarithmic increase in strain from 0.001 to 0.1, using a constant frequency of 1 Hz and constant temperature at 5 °C to determine: 1) Stiffness of the gel was determined as the storage modulus G' at $\gamma = 0.001$; 2) The strength of the gel and the end of the linear viscoelastic range (LVR) were determined as the point where the plateau value of G' starts to decrease by 3-5%. The samples were first manually stirred using a plastic spoon for approx. 10 s, to ensure a uniform and representative sample, then immediately carefully placed on the plate where the sample was allowed to settle for 1 min before measurement started. Individual samples were run in triplicates.

Microbiological analysis

After one week of cold storage, all cheese samples manufactured were tested for coliforms using Violet Red Bile (VRB) agar according (Hausler, 1972) to ensure safety for sensorial analysis.

Sensory analysis

A ranking test was performed to evaluate the overall acceptability of the fresh cheeses. Approximately 80 non-trained panelists participated in two sensory sessions and each session included two replicate blocks of fresh cheeses. Each cheese was ranked 4 times by approximately 40 random non-trained panelists.

After one-week storage at 4 °C, the sensory test was carried out. Samples were transferred into 30 mL cups and tempered to room temperature. The analysis took place in the sensory test lab associated with the Food Pilot Plant at KBM, NMBU. Six panelists operated at the same time in individual booths, and each panelist was provided with two series of coded fresh cheese samples,

water and a QR code print. Each series represented one production block and included 5 cups of the 5 MF-DF treatments. Panelists were instructed to scan the QR code which directed them to the test form and asked to wash their mouth between each sample to prevent carry over taste during the sensory evaluation. The test form was designed to guide the panelist throughout the evaluation. The panelists were asked to rank the five fresh cheese samples within each series using a scale from 1 to 5 according to their perceived preference; where 1st rank represent 'Like the most' and 5th rank represent 'Like the least'.

Statistical analysis

The entire experiment was carried out in four replicate blocks with four separate milk deliveries and successive filtrations performed. Significant effects of the DF treatments were evaluated using ANOVA at $P < 0.05$, using the DF treatment and replicate block as fixed factors. The mean values were compared using a Tukey pairwise comparison test. The sensory rank data were analyzed using a nonparametric Friedman's test, which allow the use of original ordinal ranked data and ignore additional dependency between duplicates. The output is the rank sum which is the total of the scores given by the panelists to each sample. All statistic data were processed using packages and functions in R Studio (Version 1.2.1335© 2009-2019 RStudio, Inc., Boston, MA). All significant levels were declared at $P < 0.05$.

RESULTS AND DISCUSSION

The acid-DF of the casein concentrates influenced the acidification kinetics during cheesemaking, the casein leakage to the whey as well as the rheological properties and the liking of the cheeses.

Effect of CC composition on fermentation curves

The total protein concentration of the CC showed small significant variations between the RDR and all other DF-CC in this study, Table 2, with RDR being lower than the others. As MF removes whey protein, concentration to 8 % protein increases the casein/total protein ratio to 0.91 (Gaber et al., 2020a).The Ca:Protein ratio was not however significantly influenced. Table 2 shows that DF using acidified DF-water, significantly increased the solubilization of Ca and Mg in the CC, resulting in their higher Ca^{2+} content and reduced Mg content. The fermentation kinetics of the different MF and MF-DF treatments are shown in Figure 2. The different acidifying agents used for DF-water, resulted in slightly different acidification patterns of the CC during fermentation. The reduced pH in combination with increased content of Ca^{2+} in LDR and CDR resulted in a faster fermentation profile compared to MFR. Citrate ions in CDR with high binding affinity to Ca^{2+}, causes a shift of the buffering capacity towards a high or neutral pH, due to increased content of partially solubilized colloidal inorganic phosphate (Metzger et al., 2000, Salaün et al., 2005). Therefore, the buffering capacity was assumed to be less throughout the fermentation process of CDR, which achieved a faster acidification compared with fermentation of the other DF retentates. As DF with non-acidified water reduces the buffering capacity in UF retentates (St-Galais et al., 1992), DF probably also contributed to an improved acidification kinetic of the RDR as compared with MFR in our study. According to findings by Gaber et al. (2020a), differences in the Ca^{2+}, P and Pi concentrations as an effect of the different DF-techniques would be expected. The absence of these findings in this current experiment is possibly attributed to the scale up of the volumes in the process, to differences in the types of equipment used or to a combination of the two. In the previous experiment the DF was performed using a 500kDa cellulose membrane, while in the current study, DF was performed using the same 0.14-µm MF ceramic membrane as used for the

initial MF concentration. These two membranes differ both in type of material and in pore size, with the latter being more open. Our results are, however, in accordance with the findings of Ferrer et al. (2014), who showed that significant decreases in the concentration of total Ca, P and P_i were only observed at a DF factor of 50 and above, which is a higher DF factor than what was used in this study (30).

Cheese and whey compositional analysis

The electropherograms of the cheese whey, Figure 3, shows that MFR cheese lost a substantial amount of casein to the whey. MFR cheese whey had a significantly ($P < 0.05$) higher peak area of α_{s1}-CN, β-CN and κ-CN compared to DF (RDR, LDR, CDR and ODR) cheese whey. Between the DF-treated CCs, RDR had the least casein leakage to the whey during cheese drainage, while CDR, ODR and LDR gave a higher casein leakage to the whey. Whey from all cheeses made from DF-treated retentate had no α_{s2}-CN content. The peak area of α-lactalbumin and β-lactoglobulin were also higher in MFR whey compared to whey from DF-cheeses. Consequently, DF cheese had higher total protein content as compared with MFR cheese, this was significant for RDR and ODR cheese, Table 3.

The concentration of total P in the MFR cheese whey was significantly higher than in the DF cheese whey, Table 3, this is most probably correlated with the high loss of casein to the MFR cheese whey. The Ca:Protein ratio was similar for all DF cheeses, Table 3, while MFR cheese had an increased Ca:Protein ratio. The combined effect of diafiltration and pH reduction could be an explanation for an increase in the Ca:Protein ratio of MFR with a relatively high pH (4.9) compared to DF-cheese with lower pH (4.8). The low Mg, Na and K content in the DF cheeses compared to

MFR cheese, Table 3, is a result of their initially reduced content in the MF retentate followed by a further dilution through the DF treatment. The change in the mineral balance, together with the reduced lactose content through the MF-DF process, resulted in an altered fermentation characteristics of the DF retentates. Nevertheless, the lactic acid content of the cheeses was not significantly different, indicating that the lactic acid production by the starter culture was not influenced by the DF treatments. In addition, all DF retentates had a pH of 4.7 after 17h of fermentation.

Rheological properties of fresh cheese

Representative strain sweep plots for fresh cheese are shown in Figure 4. DF significantly (P<0.05) increased the storage modulus (G') of the fresh cheeses compared with MFR. RDR, followed by CDR, then ODR had the highest G' and gel strength (τ; 4.4, 2.3, 2 Pa, respectively) compared with LDR (τ; 0.8 Pa) and MFR (τ; 0.07 Pa). Consequently, the LVE range of DF fresh cheeses varied: RDR, CDR and ODR cheeses could handle a strain sweep from 0.5 to 0.7 before deformation occurred, while a lower strain range of max 0.1 deformed LDR and MFR cheeses. Ferrer et al. (2014) showed that increasing the DF factor from 50 to 100 reduced the storage modulus of gels. In contrast to the findings of Ferrer et al. (2014), our results showed that DF (30 DF) improved the storage modulus of the cheeses. This may be due to the increased WP:CN ratio of RDR and CDR compared MFR (Gaber et al., 2020a). Nevertheless, the higher gel strength of CDR cheese compared to LDR cheese, are in accordance with the findings of others. Shehata et al. (1966) observed firmer blue cheese texture when combining lactic culture with citric acid than with lactic acid. Farkye et al. (1995) reported firmer fresh cheeses (Queso blanco) by direct acidification using citric acid rather than lactic acid. Schäfer et al. (2019) reported firmer texture

of fresh cheese produced by MF retentate pre-acidified with citric acid as compared to using non-pre acidified MF retentate. This may be due to the high binding affinity of citrate to calcium which may contribute or enhance the ability of the casein-casein binding during the gel network formation rather than being as free ions. Similarly, Dagostin et al. (2012) obtained firmer cheese when using CO_2 for pre-acidification of the milk compared to lactic acid. Although the MFR and the MF-DF CC were submitted to same heat-treatment (73 °C for 15 s) prior to cheesemaking, the heat treatment might not have been sufficient to result in significant WP denaturation (75 °C and above) for their further contribution to the MFR gel network. Protein concentration, WP to casein ratio, ionic strength and concentration of calcium in the serum phase, influence the gel formation properties and rheological characteristics of the acidified gel (Mistry and Maubois, 2004, Lucey, 2016). Therefore, the reduced casein and WP loss in whey of MF DF cheeses and the reduced Ca: Protein content in the MF DF cheeses improved the gel network formation and increased the *G'* value.

Sensory test of fresh cheeses

Figure 5 present the sum of ranks given by all panelists for each experimental cheese. The ranking test showed that CDR cheese ranked significantly (P<0.05) highest as "Like the most". The remaining DF fresh cheeses (RDR, LDR and ODR) was ranked higher in liking compared to MFR, which ranked significantly (P< 0.05) as "liked the least". Citric acid degradation by starter culture plays a major role in the production of fresh cheese flavor (Urbach, 1997, McSweeney et al., 2017). The culture used contain citrate degrading lactic acid bacteria which produces the buttery flavor (diacetyl) found in the CDR sample. Other studies confirm the effect of citric acid on the flavor development, and Schäfer et al. (2019) showed that pre-acidification of MF retentate with citric acid for fresh cheese production reduced the bitterness level compared to non-pre acidified fresh

cheese. When comparing sensory preference of pre-acidified Queso Blanco cheese, Farkye et al. (1995) also reported that cheese pre-acidified with citric acid were favored over lactic acid. Thus, the most liked ranking of the CDR cheese in our study could be attributed to its flavor, along with its elastic texture (Figure 4), when compared to the other DF cheeses. Whereas the very weak texture for the MFR cheeses was most probably the reason for its "the least liked" rank. A high elastic modulus of the fresh cheese seemed to be necessary for a good sensorial rank. Of all the fresh cheeses produced from acidified DF in our study, CDR showed the highest elastic modulus. However, RDR (fresh cheese from MF DF using non-acidified DF) had even higher elastic modulus, yet the liking rank sum of this cheese indicated an average perception.

CONCLUSION

Production of acid coagulated fresh cheese from MF concentrates benefit from the introduction of acidified DF water to overcome challenges related to fermentation time, protein loss in whey and a subsequently weak texture. Cheese produced from 8 % MF concentrate using acid DF with citric acid achieved faster fermentation, firmer texture and better sensory perception compared with cheese made from 8 % MF concentrates produced using lactic acid or carbonation for acidification of the DF water. This however does not exclude their potential usage in other fermented dairy products. Lactic acid or carbonation may be considered for production of fermented dairy products from MF retentate where a lower elasticity is desired.

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Table 1. Abbreviations of the different treatments.

Abbreviation	Meaning
MFR	Products obtained from MF process
RDR	Products obtained from MF-DF with just water
LDR	Products obtained from MF-DF with water acidified with lactic acid
CDR	Products obtained from MF-DF with water acidified with citric acid
ODR	Products obtained from MF-DF with water acidified with carbonation (CO_2)

Table 2. Compositional analysis of skim milk (SM) and casein concentrates (starting material for cheesemaking); MF retentate (MFR) and diafiltration (DF) retentates.

Constituents	SM		MFR		Retentate[1] RDR		LDR		CDR		ODR	
pH	6.67	±0.01	6.64 ^{a}	±0.04	6.64 ^{ab}	±0.09	6.45 ^{b}	±0.05	6.52 ^{b}	±0.06	6.60 ^{b}	±0.06
Composition (%)												
Total Protein	3.4	±0.1	8.3^{a}	±0.1	7.9^{b}	±0.5	8.4^{a}	±0.1	8.2^{a}	±0.3	8.4^{a}	±0.1
Lactose	5.1	±0.3	4.3 ^{a}	±0.3	3.5 ^{b}	±0.2	3.5 ^{b}	±0.2	3.3 ^{b}	±0.0	3.4 ^{b}	±0.1
Minerals[2] (mM)												
Ca	28.6	±2.4	69.8 ^{a}	±0.1	69.8 ^{a}	±0.1	69.8 ^{a}	±2.0	69.8 ^{a}	±0.1	69.2 ^{a}	±1.2
Ca^{2+}	2.1	±0.1	2.5^{b}	±0.2	2.8^{a}	±0.1	3.2^{a}	±0.3	3.3^{a}	±0.1	2.8^{a}	±0.1
P	30.2	±2.6	62.9 ^{a}	±1.8	61.3 ^{a}	±0.1	61.3 ^{a}	±0.1	61.3 ^{a}	±0.1	61.3 ^{a}	±0.1
Pi	29.2	±4.0	51.3 ^{a}	±4.6	42.5 ^{a}	±6.5	46.9 ^{a}	±3.3	49.2 ^{a}	±4.5	49.3 ^{a}	±3.4
Mg	4.5	±0.4	7.4 ^{a}	±0.1	6.9^{b}	±0.1	6.7^{b}	±0.2	6.7^{b}	±0.2	6.7^{b}	±0.2
K	42.8	±4.8	47.3 ^{a}	±1.4	39 ^{b}	±1.2	38.3 ^{b}	±0.1	38.3 ^{b}	±0.1	38.3 ^{b}	±0.1
Na	14.2	±1.4	15.7 ^{a}	±0.2	13 ^{b}	±0.1	12.8 ^{b}	±0.2	12.9 ^{b}	±0.2	12.8 ^{b}	±0.2
Ca/Na	2.0	±0.1	4.4 ^{b}	±0.1	5.3 ^{a}	±0.1	5.4 ^{a}	±0.1	5.3 ^{a}	±0.1	5.3 ^{a}	±0.1
Ca/K	0.6	±0.1	1.4 ^{b}	±0.1	1.7 ^{a}	±0.1	1.8 ^{a}	±0.1	1.8 ^{a}	±0.1	1.8 ^{a}	±0.1
Ca: Protein (mol g^{-1})	8.4 ^{a}		8.3 ^{a}		8.8 ^{a}		8.2 ^{a}		8.5 ^{a}		8.2 ^{a}	
Ca: Protein (mg g^{-1})	33.7 ^{a}		33.4 ^{a}		35.4 ^{a}		33.1 ^{a}		34.0 ^{a}		32.9 ^{a}	
Organic acid (mmol/l)												
Lactic acid	n.d.	±0.0	n.d. ^{b}	±0.0	0.2 ^{b}	±0.4	1.8 ^{a}	±1.1	0.3 ^{b}	±0.3	0.4 ^{ab}	±0.9
Citric acid	11.7	±1.3	10.5 ^{a}	±1.0	9.2 ^{bc}	±0.6	9.2 ^{bc}	±0.8	9.8 ^{ab}	±0.5	8.7^{c}	±0.4

$^{a–b}$ Means within a row with different superscripts differ according to Tukey's pairwise comparison ($P < 0.05$) (SM not included in the statistical analysis)

n.d.: not detected (below threshold value for analysis)

[1]Retentate from MF-DF treatments with: Water (RDR); water acidified with lactic acid (LDR); water acidified with citric acid (CDR); water acidified with CO_2 (ODR)

[2]Minerals: Ca= Calcium, Ca^{2+} = Ionic calcium, P= Phosphate, P_i= Inorganic phosphate, Mg= Magnesium, K= Potassium, Na= Sodium

Table 3. Compositional analysis of acid coagulated fresh cheese and cheese whey made from MF retentate (MFR) or different acid-diafiltration (DF) retentates.

Constituents	Cheese[1]									
	MFR		RDR		LDR		CDR		ODR	
pH	4.85	±0.00	4.76	±0.04	4.76	±0.06	4.75	±0.07	4.76	±0.01
Composition (%)										
Total Protein	9.1^{c}	±0.2	9.8^{ab}	±0.3	9.4^{bc}	±0.8	9.4^{bc}	±0.3	10^{a}	±0.7
Lactose	3.4^{a}	±0.3	2.1^{b}	±0.4	2.1^{b}	±0.4	2.1^{b}	±0.1	2.3^{b}	±0.2
Total Solids	14.3^{a}	±0.1	14^{ab}	±0.5	13.3^{b}	±1.4	13.4^{ab}	±0.3	14^{ab}	±0.5
Yield	79.2^{a}	±4.2	73.9^{a}	±6.1	79.7^{a}	±3.9	77.3^{a}	±1.0	75.9^{a}	±4.2
Minerals (mM)										
Ca	73.4^{a}	±1.3	67.8^{a}	±8.1	71.1^{a}	±4.9	69.6^{a}	±2.7	71.7^{a}	±2.9
P	67.1^{a}	±1.2	65.6^{a}	±4.0	65.1^{a}	±4.4	65.3^{a}	±2.1	67.5^{a}	±2.4
Mg	7.8^{a}	±0.1	6.6^{b}	±0.7	6.9^{b}	±0.4	6.7^{b}	±0.2	7^{b}	±0.2
K	49.5^{a}	±1.4	36.9^{b}	±4.4	38^{b}	±2.6	37.6^{b}	±1.3	38.5^{b}	±1.8
Na	17.0^{a}	±0.2	12.8^{b}	±1.2	13.3^{b}	±0.7	12.9^{b}	±0.2	13.4^{b}	±0.4
Ca: Protein (mol g^{-1})	8.2^{a}		6.8^{b}		7.3^{b}		7.3^{b}		7.1^{b}	
Ca: Protein (mg g^{-1})	32.1		27.5		29.5		29.4		28.6	
Lactose and Organic acids (mmol/l)										
Lactic acid	168.2^{a}	±5.5	164.1^{a}	±14	155.5^{a}	±15.4	165.3^{a}	±11.8	170.7^{a}	±20.2
Citric acid	0.4^{a}	±0.1	0.7^{a}	±0.5	1.1^{a}	±0.9	1.3^{a}	±1.1	0.5^{a}	±0.2
Acetic acid	16.8^{a}	±1.1	12.4^{b}	±0.7	11.6^{b}	±1.6	12.9^{b}	±2.2	13.3^{ab}	±1.0
	Whey[1]									
	MFR		RDR		LDR		CDR		ODR	
Composition (%)										
Total Protein	4.3^{a}	±1.7	1.4^{b}	±0.5	1.6^{b}	±0.4	1.2^{b}	±0.1	1.4^{b}	±0.1
Lactose	3.7^{a}	±0.2	2.5^{b}	±0.6	2.3^{b}	±0.3	2.1^{b}	±0.2	2.4^{b}	±0.1
Minerals[2] (mM)										
Ca	70.4^{a}	±6.2	64.2^{a}	±6.2	66.7^{a}	±4.7	64.8^{a}	±4.3	66.1^{a}	±5.9
Ca^{2+}	24.5^{a}	±1.02	24.5^{a}	±1.8	25.08^{a}	±0.5	24.4^{a}	±0.9	25.4^{a}	±0.8
P	50^{a}	±5.5	37.9^{b}	±4	41.1^{b}	±4	37.6^{b}	±3.7	40.3^{b}	±3.2
Pi	39.5^{a}	±7.3	44.1^{a}	±2.9	43^{a}	±7.9	41.8^{a}	±7.4	43.6^{a}	±8.7
Mg	7.8^{a}	±0.5	6.3^{a}	±0.7	6.7^{a}	±0.7	6.3^{a}	±0.6	6.7^{a}	±0.7
K	49.8^{a}	±4.4	39^{ab}	±6.3	38.3^{b}	±2.0	34.9^{b}	±2.9	39^{ab}	±3.8
Na	18.2^{a}	±2.1	14.7^{ab}	±1.8	14.9^{ab}	±2.0	14^{b}	±0.2	15.7^{ab}	±0.8
Ca: Protein (mol g^{-1})	20.1^{b}		51.7^{a}		41.3^{a}		49.6^{a}		46.8^{a}	
Ca: Protein (mg g^{-1})	0.6		1.8		1.4		1.7		1.6	
Lactose and Organic acids (mmol/l)										
Lactic acid	149.7^{a}	±8.3	139.2^{a}	±14.9	131.4^{a}	±5.6	128.1^{a}	±21.12	132.2^{a}	±12.6
Citric acid	1.4^{a}	±0.9	2.3^{a}	±0.8	2.5^{a}	±1.4	2.6^{a}	±0.9	1.8^{a}	±0.2
Acetic acid	14.8^{a}	±1.4	10.6^{b}	±2.1	9.4^{b}	±1.3	9.5^{b}	±1.9	10.5^{b}	±0.9

$^{a–b}$ Means within a row with different superscripts differ according to Tukey's pairwise comparison ($P < 0.05$)
[1]Made from MF CC: Reference with no DF (MFR) and MF-DF treatments: Water (RDR); DF water acidified with lactic acid (LDR); DF water acidified with citric acid (CDR); DF water acidified with carbonation (ODR).
[2]Minerals: Ca= Calcium, Ca^{2+} = Ionic calcium, P= Phosphate, P_i= Inorganic phosphate, Mg= Magnesium, K= Potassium, Na= Sodium

Table of figures

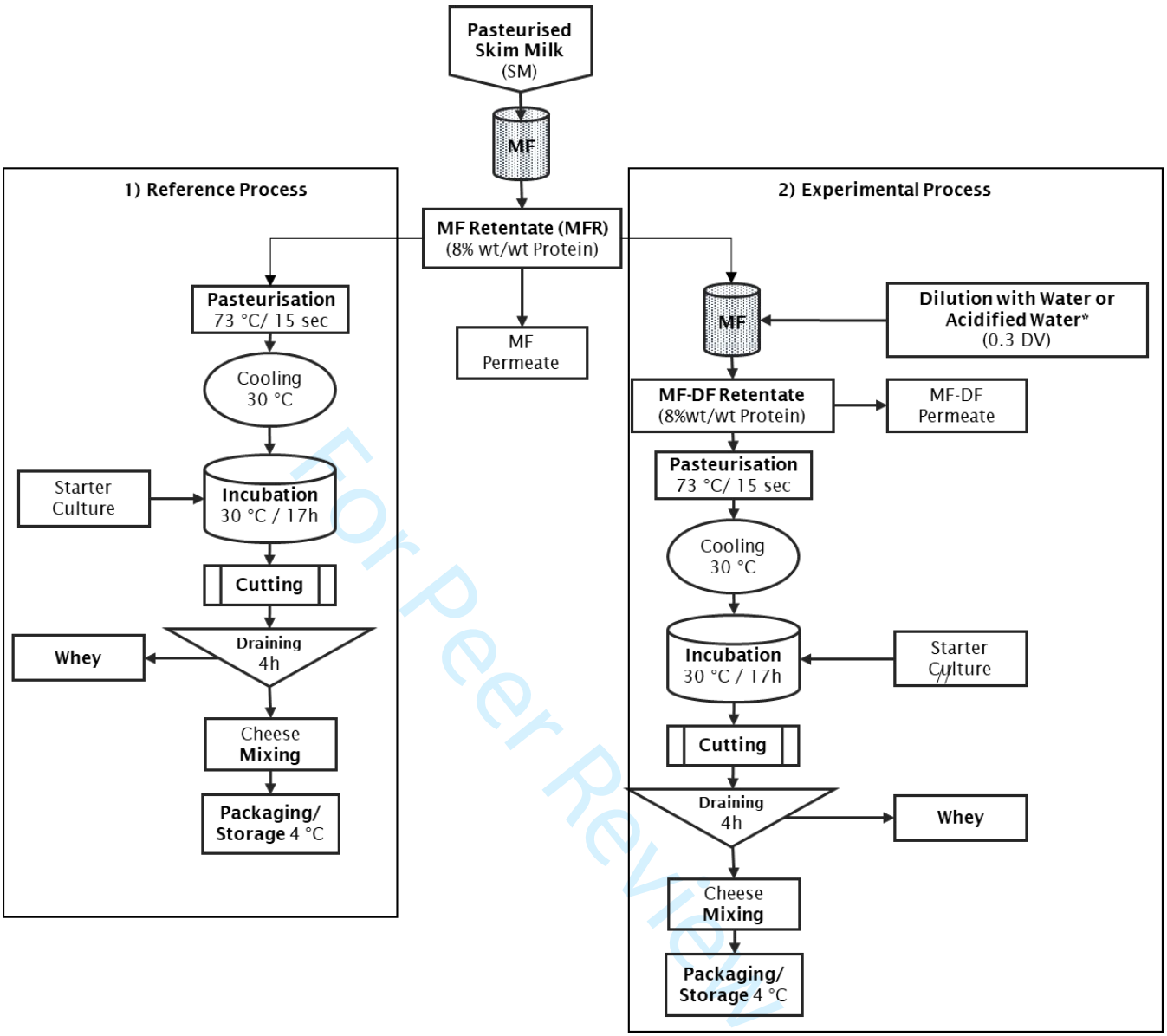

Figure 1 Process flow chart for manufacture of acid coagulated fresh cheese. 1). From microfiltration (MF) casein concentrates 8% wt/wt protein (reference fresh cheese), and 2). From diafiltered (DF) MF casein concentrate 8% wt/wt protein (Experimental fresh cheeses). *DF was done by use of water or acidified water (by use of lactic acid, citric acid or carbonation)

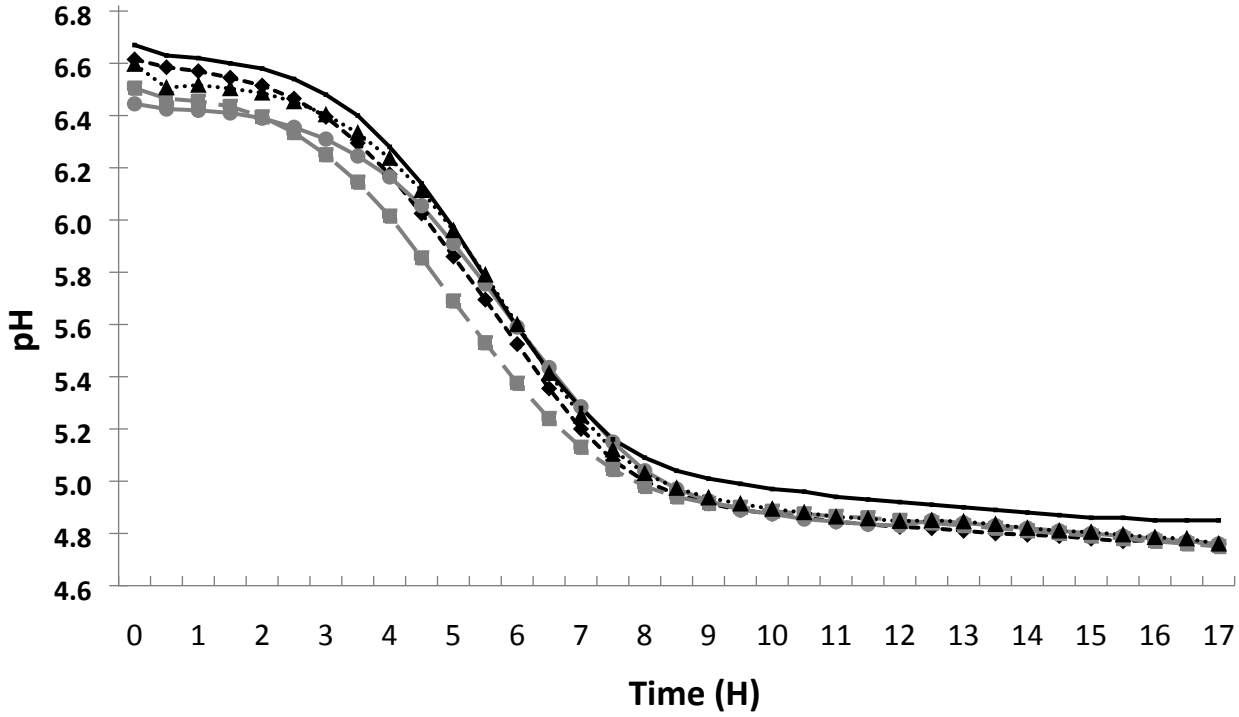

Figure 2 Fermentation curves of acid coagulated fresh cheese made from casein concentrates obtained by: MF (MFR) (——); MF-DF-water (RDR) (--♦--); MF-DF- water acidified with citric acid (CDR) (—■—); MF-DF- water acidified with lactic acid (LDR) (—●—) and MF-DF- water acidified with CO_2 (ODR) (⋯▲⋯)

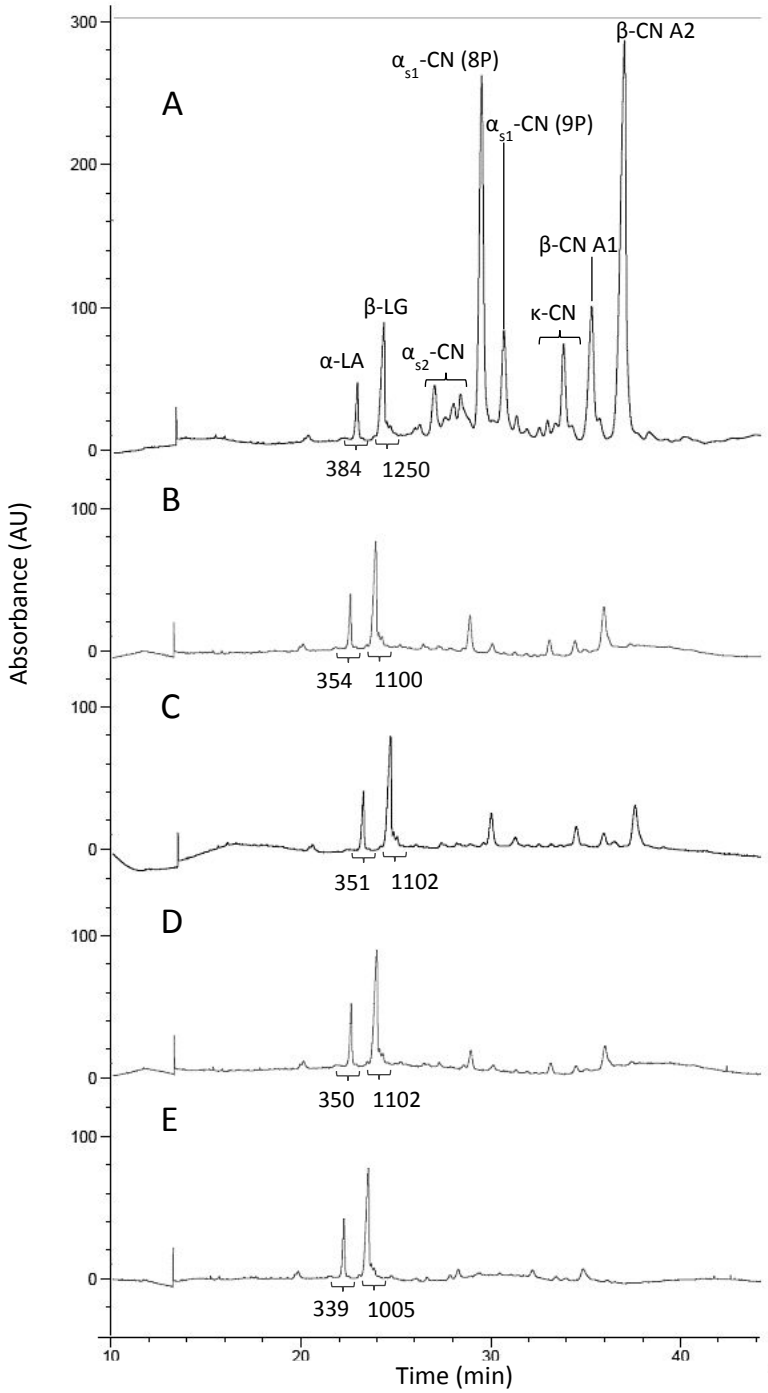

Figure 3 Electropherograms of cheese whey from MF casein concentrates (A) MFR (no diafiltration (DF)), (B) LDR (DF water acidified with lactic acid), (C) ODR (DF water acidified with CO_2), (D) CDR (DF water acidified with citric acid), (E) RDR (DF-water). The peak area of the α-lactalbumin and β-lactoglobulin is indicated below the peak in each diagram.

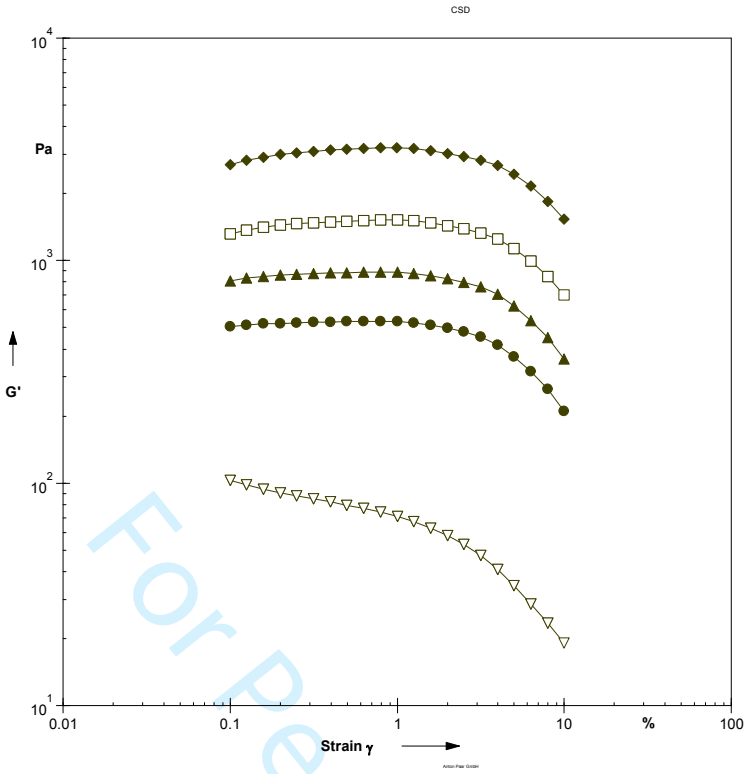

Figure 4 Representative plot of storage modulus (*G*') as a function of strain amplitude sweep for acid coagulated fresh cheeses made from MF casein concentrates: (♦) DF-water (RDR); (□)DF water acidified with citric acid (CDR); (▲) DF water acidified with CO_2 (ODR); (●) DF water acidified with lactic acid (LDR); (▽) no DF (MFR).

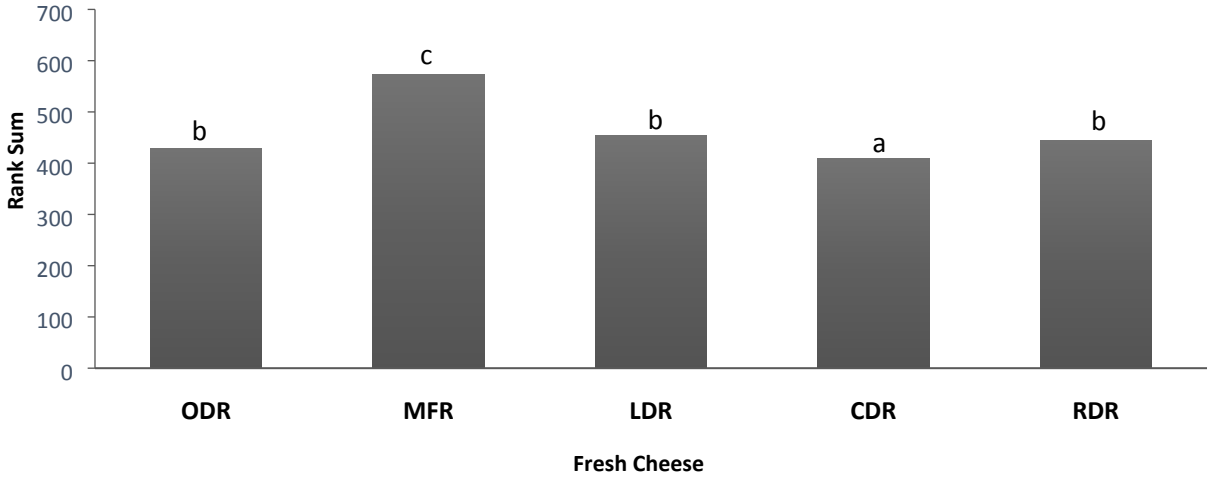

Figure 5 Rank sum plot for sensory test given by approx. 80 non-trained panelist per experimental factor (fresh cheese) for 4 replicate blocks. Fresh cheese ranked significantly different (P<0.05) are indicated with different letters. MFR (MF-casein concentrates cheese), LDR (DF-lactic acid cheese), ODR (DF-CO2 cheese), CDR (DF-citric acid cheese), RDR (DF-water cheese). On a scale of five, 1st ranked the most liked and 5th ranked the least like, cheese with the lowest rank sum value represent the best score of 'Like the most'.

Response to editor and reviewers:
The authors wish to thank the editor and the reviewers for valuable comments and suggestions on the manuscript.
The authors have done their best to answer all reviewers' comments and adjust the manuscript accordingly.

Reviewer(s)' Comments to Author:

Reviewer: 1

This short paper describes a series of studies of the impact of acidification of diafiltration water on the characteristics of acid coagulated fresh cheese made from casein concentrates made using the modified separation process. The work is straightforward, the paper well written, and the topic of interest, as the effects seem quite notable (especially around enhanced casein retention and impact on subsequent cheese characteristics). So, I am happy to recommend it for publication in JDS, subject only to some minor considerations as follows:

• L(ine)71-3: awkward link between sentences
AU: Sentences rephrased

• L77: when studies of whey protein removal are discussed and compared to the current work, it should be clarified where the samples were heated (and hence the issue is whey protein denaturation as opposed to just the proteins being present). The impact of whey proteins in the current study where heat treatments were not sufficient to result in significant denaturation is interesting.
AU: The sentence in L77 has been modified to include where the WP was denatured and how the insufficient denaturation of WP contributes or not to the texture. The matter has also been referred to further in the text L325 to point out this observation.

• L141-6: it is important to clarify what the water pH was in all cases, whereas at present it is only stated that the carbonation resulted in a pH of 4.6. as a small point, the ODR code for the carbonation sample is not very intuitive in terms of the meaning of the letters. Also, avoid use of vague terms like 'slightly acidified' (e.g., on L348)
AU: The pH of the acidified DF water is mentioned between brackets in the text. The sentences are now modified to make it clearer.
The authors understand that the choice of ODR code might have not been the easiest to relate CO_2 to and intuitive impression may mean oxygen for example rather than CO_2, but the letter C has been used to code citric acid already for example and it might be relatively late to change the code at this stage since the same code has been used to express the same treatment in the previously published work. The authors are happy to receive suggestion for code names.

The term ‘slightly’ has been removed where not specific or replaced by more specific term.

• L254: state what stream reduced minerals mentioned were found in
AU: Done

Reviewer: 2

It was difficult to read with all of the abbreviations, so I made a cheat sheet to refer to. Maybe a Table with all the abbreviations on the DF milks.
AU: A Table (1) has been added to the revised version.

Table 1: MF removes whey protein and I suspect the residual WP would have no real impact especially at the pasteurization temperature used. At 8 % protein that would be a substantial increase in casein/total protein ratio. Can you include this on the Table 1.
AU: The casein concentration has not been measured in this study but the previous paper Gaber et al., 2020, which this work builds on, provided details on the casein concentration for the different MF and DF treatments, and the CN/True protein ratio was/is 0.91. A sentence has been added to the results and discussion section L254 to give background info on the Casein/total protein.

When you DF with citric acid and you lose some calcium why is the cheese harder?
AU: A sentence has been added to L322 trying to answer why.

Flavor of cheese with citric acid DF? Did the cultures have a citric acid fermenter in it?
AU: Yes, the cultures used are very fast citrate fermenter and diacetyl producers. This info is now added to the text L341.

Line 292: Could a difference of 0.1 pH make that much of a difference especially since most calcium should be dissolved from casein at these pH's.
AU: The author thinks it's the combined effect of diafiltration and pH reduction rather than the pH reduction by itself. This sentence had been modified to answer this question L295.

You mentioned you measured yield but do not say anything about it. You could report moisture adjusted yield as that might give insight into the impact of loss of protein in cheese whey.
AU: The yield values are reported in Table 3 (former table 2), however the data were not discussed because of non-significant differences between the treatments.

What is rank sum (Figure 5)?
AU: The rank sum, is the total of scores given by the panellists to each sample. This info is now added to the material and methods section L243.

Sensory liking is not the same as liked? Do you have any more insight into flavor profiles.
AU: Sensory liking is the same as liking, but we wanted to specify it is related to the sensory test. The word sensory is now removed from the revised version.
We have not carried out descriptive sensory test to have insight into the flavour profiles, the textures of the samples were too different, and this would have interacted with a sensory

profiling focused on flavour. Therefore, that was not done. It will be very interesting to have a closer look into it in the future.

The conclusions read too much like an abstract.
AU: The conclusion is improved in the revised version.

Reporting checklist for JDS for primary research that does not involve animals. Adapted from the REFLECT and STROBE-Vet statements. For intervention trials or observational studies in humans, consider using CONSORT or STROBE.

Indicate where in the paper these items are reported

Paper section and topic	Item	Descriptor of statement item	Reported on Page #
Title & Abstract	1	Describe how experimental units were allocated to treatments (e.g., "random allocation," "randomized," or "randomly assigned"), or whether the study was observational, or an assessment of a method. Clearly state whether the outcome was the result of natural exposure or was the result of a controlled experiment. For observational studies, include a common study design term.	1, 3
Introduction Background	2	Provide scientific background and explanation of rationale.	4
Methods Participants	3	If relevant, describe the eligibility criteria for human participants (e.g., in sensory evaluation or in surveys) and the settings and locations where the data were collected.	11
Interventions	4	Describe precise details of the interventions (treatments) for each group, the level (e.g., farm, animal, batch, or product) at which the intervention was allocated, and how and when interventions were administered.	11-12
Objectives	5	State specific objectives and hypotheses. Clearly state primary (i.e., the one that determined the sample size) and secondary objectives (if applicable).	7
Outcomes	6	Clearly define primary and secondary outcome measures and the levels at which they were measured, and, when applicable, any methods used to enhance the quality of measurements (e.g., multiple observations, training of assessors).	12-17
Sample size	7	Explain how sample size was determined and, when applicable, explain any interim analyses Where relevant, include sample size determinations at each level of the organizational structure and how any non-independence among groups or samples within a group were accounted for.	7
Randomization -- Sequence generation	8	Describe the method used to generate the random allocation scheme, including details of any restrictions (e.g., blocking, stratification)	
Randomization -- Allocation concealment	9	Describe the method used to implement random allocation, including how treatment assignment was concealed (e.g., treatments may be randomly assigned, but if study units or samples are labelled with letters or colors, differentiation between groups is not concealed).	

			Reported on page #
Randomization -- Implementation	10	Describe who generated the allocation sequence, who enrolled study units, and who assigned study units to their groups at the relevant level of the organizational structure.	
Blinding (masking)	11	State whether or not those administering the interventions and those assessing the outcomes were blinded to group assignment; if done, how the success of blinding was evaluated. Provide justification for not using blinding if it was not used.	
Analytical methods	12	Describe the analytic methods in sufficient detail for replication. Provide data or references to validate the accuracy of the methods under the conditions of this study. Include measures of the precision (repeatability) of assays and the limits of quantification. In sensory evaluations of foods, report the individual traits assessed, not only aggregated scores. For identification, or description of the properties of biological compounds, report the composition of compounds and the techniques by which they were determined. Describe and support how the techniques were validated.	**8-11**
Statistical methods	13	Specify the statistical methods used to compare groups for all outcomes. Clearly state the level of the data on which statistical analysis were performed i.e., show that the correct degrees of freedom were employed for the structure of the data. Clearly state if repeated measures of the outcome were made and how this was accounted for in the statistical analyses. Clearly state all covariates tested.	**12**
Results Study flow	14	Account for the flow of study units through each stage of the study and analysis (a diagram is recommended). Specifically, for each group, report the numbers of study units randomly assigned or enrolled, receiving intended treatment, completing the study protocol, and analyzed or excluded. Describe any deviations from the study protocol as planned, and the reasons for these changes.	**12-17**
Contextual data	15	Where human evaluators (e.g., sensory analysis) or subjects are involved, describe the demographic and other relevant characteristics of participants.	
Numbers analyzed	16	Specify the number of study units (denominator) in each group included in each analysis and whether the analysis was by "intention-to-treat" or whether units were excluded if they did not comply with the intended treatment. State the results in absolute numbers when feasible (e.g., 10/20, not 50%).	
Outcomes	17	For each primary and secondary outcome, provide a summary of results for each group, accounting where relevant for each relevant level of the organizational structure, and the estimated effect size and its precision (e.g., 95% confidence interval). Where relative measures of effect are reported, also provide absolute values.	**12-17**
Ancillary analyses	18	Address multiplicity by reporting any other analyses performed, including subgroup analyses and adjusted analyses, indicating which were pre-specified and which were exploratory.	

			Reported on page #
Discussion Interpretation	19	Provide interpretation of the results, taking into account the study hypotheses, and sources of potential bias or imprecision, including explicit discussion of multiplicity of analyses and outcomes. Explicitly discuss the strengths and limitations of the study. Discuss both direction and magnitude of any potential bias. Place the results in the context of relevant literature and state whether or how the findings should change practice.	12-17
Generalizability	20	Discuss generalizability (external validity) of the study findings.	
Transparency	21	List the sources of funding for the work and acknowledge any potential conflicts of interest that the authors have.	18