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Effect of milk centrifugation and incorporation of high-heat-treated centrifugate on the composition, texture, and ripening characteristics of Maasdam cheese

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ABSTRACT

This study investigated the effect of centrifugation (9,000 × *g*, 50°C, flow rate = 1,000 L/h), as well as the incorporation of high-heat-treated (HHT) centrifugate into cheese milk on the composition, texture, and ripening characteristics of Maasdam cheese. Neither centrifugation nor incorporation of HHT centrifugate into cheese milk had a pronounced effect on the compositional parameters of any experimental cheeses, except for moisture and moisture in nonfat substance (MNFS) levels. Incorporation of HHT centrifugate at a rate of 6 to 10% of the total milk weight into centrifuged milk increased the level of denatured whey protein in the cheese milk and also increased the level of MNFS in the resultant cheese compared with cheeses made from centrifuged milk and control cheeses; moreover, cheese made from centrifuged milk had ∼3% higher moisture content on average than control cheeses. Centrifugation of cheese milk reduced the somatic cell count by ∼95% relative to the somatic cell count in raw milk. Neither centrifugation nor incorporation of HHT centrifugate into cheese milk had a significant effect on age-related changes in pH, lactate content, and levels of primary and secondary proteolysis. However, the value for hardness was significantly lower for cheeses made from milk containing HHT centrifugate than for other experimental cheese types. Overall, centrifugation appeared to have little effect on composition, texture, and ripening characteristics of Maasdam cheese. However, care should be taken when incorporating HHT centrifugate into cheese milk, because such practices can influence the level of moisture, MNFS, and texture (particularly hardness) of resultant cheeses. Such differences may have the potential to influence subsequent eye development characteristic, although no definitive trends were observed in the present study and further research on this is recommended.

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INTRODUCTION

Various milk pretreatment methods have been applied before cheesemaking to enhance quality, consistency, and functionality of different cheese varieties (Kelly et al., 2008; Johnson, 2017). Centrifugation of milk using a special centrifuge (also called Bactofuge, Alfa Laval, Richmond, VA) at a centrifugal force of ∼9,000 × *g* (at 50°C) is a pretreatment method widely used by the cheese industry for removal of *Clostridium* spores before cheesemaking. After centrifugation, milk is divided into 2 streams, namely (1) centrifuged milk containing low bacterial cells and spores count, which account for ∼97% of the feed volume, and (2) centrifugate containing high bacterial cells and spores count, which account for ∼3% of the feed volume (Kosikowski and Mistry, 1990).

Some cheese producers apply high heat treatment to the centrifugate to inactivate bacterial cells and spores and recycle the stream back into centrifuged milk before cheesemaking to minimize protein losses, as it contains ∼7% protein (Kosikowski and Mistry, 1990). High heat treatment of milk results in denaturation of whey proteins (Rynne et al., 2004), which can form complexes with whey proteins (in the serum phase) and casein micelles (Donato and Guyomarc'h, 2009). Such complexes are believed to hinder the aggregation of destabilized casein micelles during rennet-induced coagulation of milk (Vasbinder et al., 2003), and thus reduce the ability of the gels to undergo syneresis, leading to cheese curd with higher levels of moisture and moisture in nonfat substance (**MNFS**). Moisture in the cheese matrix acts as a plasticizer between the protein strands and softens the cheese texture (Lamichhane et al., 2018a). Moreover, the higher moisture and MNFS content within the cheese matrix can enhance the microbial and enzymatic activities (Beresford et al., 2001), which can alter the ripening characteristics of cheese (Rynne et al., 2004, 2007).

Some *Clostridium* spp. have been reported to be associated with late blowing defect of cheese, which is manifest as production of gas (e.g., CO_2 and H_2) and formation of high levels of butyric acid, resulting in downgraded cheeses (Klijn et al., 1995; Le Bourhis et al., 2007; Garde et al., 2011). Although the effect of centrifugation on efficacy of removal of *Clostridium* spores from milk and late blowing defect of cheese have been a research focus for several studies (Langeveld, 1971; Su and Ingham, 2000), its effect on composition, texture, and ripening characteristics of cheese has to date received little attention. As well as removal of *Clostridium* spores from milk, centrifugation also removes indigenous milk bacterial cells and somatic cells from milk by ~87 and 75 to 95% of the total count, respectively (Te Giffel and Van Der Horst, 2004; Wieking, 2004).

Maasdam is a brine-salted, large-eye forming, semihard cheese combining the traits of both Swiss and Dutch-type cheeses. Both lactic and citric acid fermentation occur during the first 24 h of manufacture and propionic acid fermentation occurs during warm-room ripening. Very little research has been published on the physicochemical properties and ripening characteristics of Maasdam and similar cheese types, such as Jarlsberg.

The aim of our study was to evaluate the effect of (1) centrifugation and (2) the incorporation of the high-heat-treated (**HHT**) centrifugate into cheese milk on the composition, pH, primary and secondary proteolysis, lactic acids levels, and texture of Maasdam cheese during ripening. In our study, centrifugation refers to the separation of bacteria and spores at a centrifugal force of ~9,000 × *g* (at 50°C with a flow rate of 1,000 L/h), whereas centrifugal separation refers to separation of milk into cream and skim milk. A parallel study was conducted investigating the effect of milk centrifugation and incorporation of HHT centrifugate on microbial composition and the levels of volatile organic compounds of Maasdam cheese (Lamichhane et al., 2018b).

MATERIALS AND METHODS

Milk Supply and Treatments

Raw whole milk was obtained from a local dairy company. From raw milk, 3 different cheese milk streams were prepared (Figure 1). Part of the raw milk was separated at 55°C (centrifuge disc separator, GEA Westfalia, Oelde, Germany) to give skim milk and cream. Control cheese milk (**CT**) was prepared by adding a portion of the resultant cream to skim to achieve a protein-to-fat ratio of 1.13:1. The remaining whole milk was centrifuged (Bactofuge disc separator, type: D3187M, Alfa Laval, Richmond, VA) at a centrifugal force of ~9,000 × *g* (at 50°C with a flow rate of 1,000 L/h) to provide centrifuged whole milk and centrifugate (also called sludge or bactofugate), which accounts for approximately 3 to 6% of the total milk feed. Centrifuged whole milk was then separated to give skim and cream. Centrifuged cheese milk (**CF**) was prepared by adding portions of the cream into the skim milk to achieve a protein-to-fat ratio of 1.13:1. High heat treatment (120°C for 26 s, plate heat exchanger, APV Schweig AG, Worb, Switzerland) was applied to centrifugate to inactivate spores and bacteria, and this centrifugate was combined with a portion of centrifuged cream and skim milk to produce the third cheese milk; that is, centrifuged milk containing HHT centrifugate (**CFHHT**). As the protein content of centrifugate after high heat treatment varied between 3.76 and 6.36% (wt/wt) between trials, HHT centrifugate was added to centrifuged milk on a protein basis rather than weight basis [i.e., approximately 12% (wt/wt) of the total protein was from HHT centrifugate in CFHHT milk; on weight basis, HHT centrifugate was added at a level of 6.6 to 10.3% (wt/wt), depending on the protein content of HHT centrifugate]. All cheese milk types (CT, CF, and CFHHT) were standardized to a protein-to-fat ratio of ~1.13:1 and pasteurized (72°C for 15 s) before cheese manufacture.

Cheese Manufacture

Three experimental Maasdam cheese types [i.e., cheese made from control milk (CT cheese), centrifuged milk (CF cheese), and centrifuged milk containing HHT centrifugate (CFHHT cheese)] were each manufactured on 3 different occasions in replicate cheesemaking trials over a 3-mo period. Standardized and pasteurized cheese milks were pumped into cylindrical, jacketed cheese vats. Each vat contained automated variable speed cutting and stirring equipment (APV Schweig AG). All cheese milks (380 kg/vat) were inoculated at 31°C with frozen direct vat inoculate cultures (Chr. Hansen Ltd., Cork, Ireland), including (1) mixed strains of mesophilic bacteria (C950, 18 mg/kg of milk), consisting of *Lactococcus lactis* ssp. *cremoris*, *Lactococcus lactis* ssp. *lactis*, and *Leuconostoc*; (2) *Lactobacillus helveticus* (LH-B01, 4.8 mg/kg of milk); and (3) propionic acid bacteria (**PAB**; PS-60, 7 mg/kg of milk). Calcium chloride (34%, wt/vol) was added at a level of 0.3 mL/kg of milk to each vat. Rennet (Chy-Max Plus, ~200 IMCU/mL; Chr. Hansen Ltd.), diluted ~1:10 with deionized water, was added at a level of 0.2 mL/kg of milk after a 40-min ripening period at 31°C. All gels were cut at a constant firmness (storage or elastic modulus, **G′**) value of 35 Pa (as measured using

a small-amplitude oscillatory rheometer, AR 2000ex, TA Instruments, New Castle, DE), and the resultant curd particle/whey mixture was allowed to heal for 7 min before being stirred continuously for another 7 min. Stirring was then stopped and a portion of whey (34 kg/100 kg of cheese milk) was removed. After whey removal, reverse osmosis water at ~50°C (23 kg/100 kg of cheese milk) was used to cook the curd to 37°C at a rate of 0.2°C/min with continuous stirring. After the curd washing and cooking steps, whey and curd were drained into a prepress vat and curds were vertically prepressed under warm whey for 25 min, with increasing pressure from 3 to 5 kPa. Whey was then drained from the prepress vat and the prepressed curd was subsequently cut into 10-kg wheels (3 wheels from each vat), placed into 10-kg molds, and pressed vertically under increasing pressure from 3.3 to 14 kPa for ~3.5 h. When the pH of the cheese curds reached 5.49 to 5.51, cheese wheels were transferred to a saturated brine solution (23% wt/wt NaCl, 0.56% $CaCl_2$, pH 5.2, and 18°C) for 24 h. After brining, cheese wheels were vacuum-packed in CO_2-permeable bags and transferred to the ripening room. The cheeses were ripened at 8°C for 10 d (preripening), at 23°C for 30 d (warm-room ripening), and finally stored at 4°C for 140 d.

Rennet Coagulation Properties

In all 3 replicate cheesemaking trials, 2 min after rennet addition and stirring, a representative sample of milk was removed from the vat and placed into a cell of a small amplitude oscillatory rheometer (AR 2000ex, TA Instruments). A concentric-cylinder measuring geometry, consisting of a cylindrical bob and cup, was used. The dynamic changes in rheology during the coagulation process were monitored using a dynamic time sweep analysis with an angular frequency of 1.0 Hz, and a strain of 0.01 at 31°C, within the linear viscoelastic region (strain <0.03) reported for rennet milk gels (Mateo et al., 2010). Total time to reach a G′ value of gels of 35 Pa (at which cutting of the gel in the cheese vat was initiated) after rennet addition was calculated.

Milk and Cheese Composition Analysis

The composition of raw milk, centrifugate, and pasteurized cheese milks were analyzed using a Fourier transform infrared spectrophotometer (MilkoScan FT 120, Foss Electric, Hillerød, Denmark). Raw milk and centrifuged milk samples were analyzed for SCC (cells/mL) with a fluoro-opto-electronic counter (Fossomatic FC, Foss). Casein number, NPN, and levels of whey protein denaturation as percentage of total whey protein of HHT centrifugate and pasteurized cheese milk samples of 1 representative trial were determined as described by Rynne et al. (2004). Grated cheese samples were analyzed at 11 d at least in duplicate for moisture, fat, protein, salt, calcium, and pH, as described by Sheehan et al. (2007a).

For determination of lactose and galactose content, cheese samples were extracted as described by Zeppa et al. (2001). Grated cheese samples (10 g) were mixed

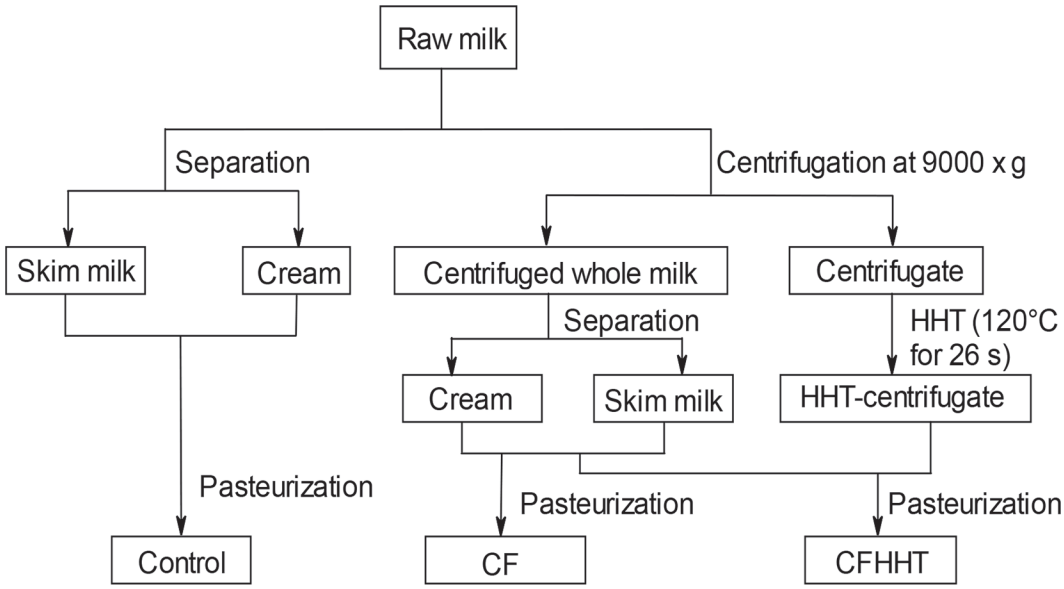

Figure 1. Flowcharts of the preparation of cheese milks [i.e., control, centrifuged (CF), and centrifuged milk containing high-heat-treated centrifugate (CFHHT)]. HHT = high heat treatment; HHT-centrifugate = high-heat-treated centrifugate.

with 50 mL of 0.013 N H_2SO_4 and stomached for 10 min using a stomacher (Iul Instruments, Barcelona, Spain) before centrifugation at 7000 × g for 5 min. The supernatant was then filtered using a 0.2-μm nylon filter. The extracted samples were then analyzed by HPLC (Waters Alliance 2695 separation module, Waters, Milford, MA) with an Aminex HPX-87C Carbohydrate column 300 × 7.8 mm (Bio-Rad, Hertfordshire, UK) under the following working conditions: injection volume of, 50 μL; mobile phase of 0.009 N H_2SO_4; flow rate of 0.5 mL/min; column temperature of 60°C; and refractive index detection (Waters 2414 RI detector). Quantification of lactose and galactose was based on the external standard method as described by Hou et al. (2014).

SDS-PAGE Analysis

The individual proteins in the raw milk, centrifugate before and after HHT, and cheese milks were identified by SDS-PAGE. All milk samples were diluted, using Milli-Q water (Millipore, Billerica, MA), to a protein concentration of ~6 μg/μL. A portion of the diluted samples were further diluted with SDS sample buffer [NuPAGE LDS Sample Buffer (4×; Thermo Fisher Scientific, Waltham, MA), composed of lithium salt, glycerine, sulfuric acid, and monododecyl ester]. For reducing SDS-PAGE, samples were treated with dithiothreitol [NuPAGE Sample Reducing Agent (10×); concentration = 500 mmol/L] at a level of 10% (vol/vol) of the total sample volume mixture. All samples were heated at 70°C for 10 min, cooled, and loaded on SDS-PAGE gels (NuPAGE 12% Bis-Tris mini gels) at a rate of 10 μg/well before running in SDS running buffer [NuPAGE MOPS SDS Running Buffer (1×)] at constant voltage of 200 V for 50 min using Mini Gel Tank (XCell SureLock Mini, Thermo Fisher Scientific). After electrophoresis, the gels were stained as described by McCarthy et al. (2012). The SDS-PAGE gels were scanned using an Epson V700 film scanner (Epson, Suwa, Nagano, Japan). The identities of principal protein bands in the milk samples were determined using prestained protein molecular weight marker (PageRuler Prestained Protein Ladder, 10 to 180 kDa, Thermo Fisher Scientific).

pH and L- and D-Lactate Analysis

The pH of cheese samples were measured on cheese slurry prepared by mixing 20 g of grated cheese and 12 g of deionized water at different time points throughout ripening (Sheehan et al., 2007a). The sample extraction method, as outlined for lactose and galactose content, was used for D- and L-lactic acid content determination. The extracted samples were analyzed for D- and L-lactic acid content using HPLC (Waters Alliance 2695 separation module) equipped with chiral column [Chirex 3126 (D)-penicillamine, column 150 × 4.6 mm, Phenomenex, Cheshire, UK], as described by Hou et al. (2014).

pH 4.6-Soluble Nitrogen and Free Amino Acids

The levels of pH 4.6-soluble N (% of total N) and free amino acids (**FAA**) of the cheeses were measured after 1, 11, 41, 65, 97, 140, and 180 d as described by Fenelon and Guinee (2000) and Sheehan et al. (2007a), respectively.

Texture Profile Analysis of Cheese

Texture properties were analyzed by TAHDi texture profile analyzer (Stable Micro Systems, Goldalming, Surrey, UK), equipped with a 70-mm (diameter) compression plate and a 100-kg load cell. Cheese was cut into 6 cube-shaped samples (25 mm^3) using a Cheese Blocker (Bos Kaasgreedschap, Bodengraven, the Netherlands), wrapped in tin foil, and stored overnight at 4°C. Cheese samples (~4°C) were compressed to 40% of their original height in 2 consecutive bites at a rate of 60 mm/min (Henneberry et al., 2015). Texture profile analyzer parameters were calculated as previously described by Chevanan et al. (2006).

Statistical Analysis

Three experimental cheese types were each manufactured on 3 different occasions in replicate cheesemaking trials. An ANOVA, using IBM SPSS software version 24 (IBM Corp., 2016), was applied to determine the effect of treatment on milk and cheese composition. A split-plot design was used to determine the effects of treatments (centrifugation or addition of HHT centrifugate into cheese milk), ripening time, and their interactions on pH, lactic acid-to-protein ratio, lactic acid, proteolysis, and texture. Analysis for the split-plot design was carried out using the PROC MIXED procedure of SAS software version 9.3 (SAS Institute Inc., 2011). Tukey's multiple-comparison test was used for paired comparison of treatment means at a 5% level of significance. IBM SPSS software version 24 (IBM Corp., 2016) was used to perform Pearson correlation between lactate-to-protein ratio and pH.

Table 1. Composition of centrifugate and pasteurized cheese milks[1]

Compositional parameter	Centrifugate[2]		Cheese milk[2]		
	Before HHT	After HHT	CT	CF	CFHHT
Protein (%, wt/wt)	6.10[a]	5.11[a]	3.38[b]	3.29[b]	3.32[b]
Fat (%, wt/wt)	0.23[a]	0.22[a]	2.98[b]	2.90[b]	2.92[b]
Lactose (%, wt/wt)	4.52[a]	4.05[a]	4.74[b]	4.66[b]	4.64[b]
Protein/fat	—	—	1.134[b]	1.135[b]	1.137[b]
Casein number	79.22	89.72	79.56	79.16	80.69
NPN (%, wt/wt)	5.91	5.57	6.33	6.57	6.47
Native whey protein (% of total)	14.86	4.71	14.11	14.27	12.85
WPD[3] (% of total whey protein)	—	68.30	5.81	4.77	14.24

[a,b]Values within a row not sharing common superscripts differ ($P < 0.05$) in the case of protein, fat, lactose and protein/fat.

[1]HHT = high heat treatment; CT = control milk; CF = centrifuged milk; CFHHT = centrifuged milk containing high-heat-treated centrifugate.

[2]Data presented are the mean of data from 3 replicate trials for protein, fat, lactose, and protein/fat; for other parameters, data are from 1 representative trial. For parameters without superscripts, statistical analysis was not carried out because the data are from only 1 representative trial.

[3]WPD = whey protein denaturation.

RESULTS AND DISCUSSION

Raw Milk, Centrifugate, and Cheese Milk Composition

The average fat, protein, and lactose contents of raw milk used for the 3 replicate cheesemaking trials were 4.01, 3.41, and 4.73% (wt/wt), respectively. Somatic cell counts of the raw milk between trials ranged between 1.2×10^5 and 2.4×10^5 cells/mL. The SCC of milk depends on factors such as breed and parity, stage of lactation, udder health, and also individual and environmental factors, as well as management practices (Li et al., 2017; Panthi et al., 2017). In general, the SCC of milk from healthy cows is less than 2×10^5 (Li et al., 2014). The efficacy of centrifugation process ($9{,}000 \times g$ at 50°C with a flow rate of 1,000 L/h) for removal of SCC was ~95% relative to the SCC in raw milk, in close agreement with the study of Wieking (2004), who reported the efficacy of centrifugation as ~95%. It is generally accepted that the SCC of cheese milk negatively influences the cheese making and final cheese quality (Panthi et al., 2017). However, in contrast, a recent study (Li et al., 2017) suggested that the somatic cells have minimal effect per se on the cheesemaking and final cheese quality. This suggests that more research is needed to better understand the role of somatic cells in cheese quality.

The composition of centrifugate before and after HHT and cheese milks is shown in Table 1. The level of whey protein denaturation (**WPD**; as a percentage of total whey protein) in centrifugate after HHT was 68.30%. It is widely recognized that HHT denatures whey proteins; for example, Rynne et al. (2004) observed that 34% of total whey protein was denatured when milk was heated at 87°C for 26 s. The protein and lactose content of centrifugate decreased, although not significantly, after HHT. This is probably due to slight dilution with process flush water when utilizing a low volume of centrifugate (~40–45 kg) in pilot-scale processing. Although fat, protein, and lactose contents of cheese milks were not statistically different, the level of WPD was ~2.5- to 3-fold higher in CFHHT milk (14.2%) than in CT (5.8%) and CF (4.8%) milks. The low level of WPD in CT and CF milk is attributed to pasteurization (72°C for 15 s) of milk, whereas the level of WPD in CFHHT milk is attributed to both pasteurization and incorporation of HHT centrifugate. The level of WPD in pasteurized milk is in close agreement with that reported by Rynne et al. (2004).

SDS-PAGE Analysis

The individual proteins in raw milk, centrifugate before and after HHT, and cheese milks were analyzed using SDS-PAGE (Figure 2). The SDS-PAGE patterns of centrifugate before HHT were different compared with centrifugate after HHT, with a lower intensity of the bands corresponding to α-LA, β-LG, and other minor proteins, such as lactoferrin and BSA, in HHT centrifugate. Moreover, some large protein aggregates were observed at the entry of stacking gel in the HHT centrifugate, as denoted by X in Figure 2A. Similar to our result, Patel et al. (2006) also observed heat-induced large aggregates in HHT milk samples. Heat-denatured whey proteins can form complexes with themselves and with caseins, particularly κ-CN, through disulfide interchange reactions and hydrophobic forces (Jean et al., 2006; Patel et al., 2006; Kethireddipalli and Hill,

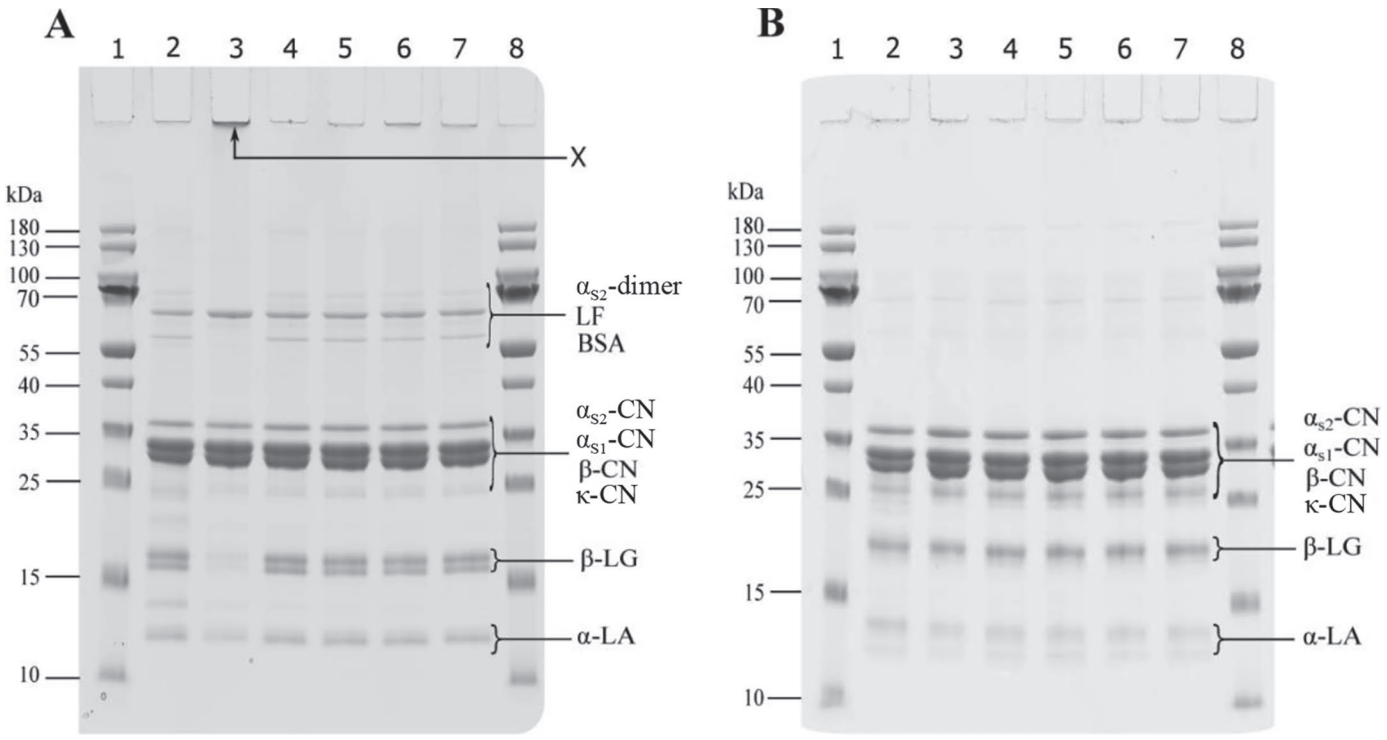

Figure 2. The SDS-PAGE patterns of milk samples under (A) nonreducing and (B) reducing conditions. Lane 1 and 8 = prestained protein molecular weight marker; lane 2 = centrifugate before high heat treatment; lane 3 = centrifugate after high heat treatment; lane 4 = raw milk; lane 5 = pasteurized (72°C for 15 s) control cheese milk; lane 6 = pasteurized centrifuged milk containing high-heat-treated (HHT) centrifugate; and lane 7 = pasteurized centrifuged milk. Protein aggregates in HHT centrifugate are denoted by X. LF = lactoferrin. Samples shown are from 1 representative trial.

2015). In reducing SDS-PAGE, the large heat-induced aggregates in HHT centrifugate disappeared when the samples were reduced with dithiothreitol; moreover, the SDS-PAGE patterns in reduced gels appeared similar (Figure 2B). This suggests that the protein aggregates in HHT centrifugate may be bonded by various molecular forces, such as hydrophobic forces and disulfide bonding (Donato and Guyomarc'h, 2009).

The SDS-PAGE patterns of raw milk and cheese milks appeared similar, although the level of denatured whey protein was higher in cheese milks, especially CFHHT, than in raw milk. This suggests that the SDS-PAGE analysis is not very sensitive for differentiating between low levels of whey protein denaturation.

Rennet Coagulation Characteristics

The average time to reach an elastic shear modulus (G′) value of 35 Pa for CFHHT, CT, and CF milks after rennet addition was 45.1, 38.7, and 40 min, respectively. Several factors can influence the rennet-induced coagulation of milk, such as milk composition and renneting conditions (e.g., pH, temperature, and ionic strength; Guinee et al., 2006; Ong et al., 2011, 2012). Some studies have reported that the heat-induced complexes formed between whey protein and κ-CN on the surface of the casein micelles inhibit the primary phase of rennet coagulation (i.e., decrease the accessibility of enzyme to κ-CN; Van Hooydonk et al., 1987). However, more recent studies have suggested that WPD has minimal effect on the primary phase of rennet coagulation; instead, WPD has a more clear effect on the secondary phase of rennet coagulation [i.e., aggregation (fusion) of destabilized casein micelles; Vasbinder et al., 2003]. The casein-whey protein and whey protein aggregates (in serum phase) can sterically hinder the aggregation of destabilized casein micelles (Waungana et al., 1996; Vasbinder et al., 2003). In the current study, although the level of denatured whey protein of CFHHT was ~2.5- to 3-fold higher than that of CT and CF milks, the rennet coagulation time of CFHHT milk did not differ statistically significantly to the others. The levels of WPD (as a percent of total whey protein) for all experimental cheese milks were below 15%, and it seems that such levels of WPD had no pronounced effect on the rennet-induced coagulation of milk. More pronounced effects were reported previously for severely heat-treated milk (Rynne et al., 2004).

Table 2. Compositional parameters and pH at 11 d of ripening in Maasdam cheeses[1]

Compositional parameter[2]	Cheese type			SEM	*P*-value
	CT	CF	CFHHT		
Moisture (%, wt/wt)	44.83[a]	44.15[a]	47.83[a]	0.72	0.057
MNFS (%, wt/wt)	58.9[a]	58.14[a]	61.78[b]	0.63	0.013
Protein (%, wt/wt)	24.04[a]	24.57[a]	23.44[a]	0.30	0.348
Fat (%, wt/wt)	23.9[a]	24.07[a]	22.61[a]	0.44	0.376
FDM (%, wt/wt)	43.31[a]	43.07[a]	43.29[a]	0.35	0.966
Salt (%, wt/wt)	1.53[a]	1.50[a]	1.73[a]	0.05	0.183
S/M (%, wt/wt)	3.41[a]	3.40[a]	3.61[a]	0.07	0.450
Total calcium (mg/100 g)	821[a]	800[a]	837[a]	12.19	0.514
pH (11 d)	5.28[a]	5.31[a]	5.27[a]	0.01	0.548
Lactose (mg/100 g)					
1 d	54.39[a]	41.82[a]	66.72[a]	16.26	0.861
11 d	20.42[a]	43.72[a]	62.63[a]	15.07	0.585
41 d	0.00[a]	0.00[a]	0.00[a]	0.00	—
Galactose (mg/100 g)					
1 d	28.09[a]	29.57[a]	35.34[a]	4.11	0.798
11 d	27.61[a]	27.26[a]	30.82[a]	4.22	0.947
41 d	0.00[a]	0.00[a]	0.00[a]	0.00	—

[a,b]Values within a row not sharing common superscripts differ ($P < 0.05$); data are the mean of data from 3 replicate trials.

[1]CT = control cheese; CF = cheese made from centrifuged milk; CFHHT = cheese made from centrifuged milk containing high-heat-treated centrifugate.

[2]MNFS = moisture in nonfat substance; FDM = fat in DM; S/M = salt-to-moisture ratio.

Cheese Composition

The composition of the experimental cheeses is shown in Table 2. Centrifugation of milk had no significant effect on mean levels of moisture, MNFS, protein, fat, salt, total calcium, lactose, and galactose of final cheeses. However, incorporation of HHT centrifugate into the centrifuged milk increased ($P < 0.05$) the mean levels of MNFS of the resultant cheeses. The average moisture content of CFHHT cheese was ~3% higher than control and CF cheeses, which was expected and sizeable in magnitude for cheese moisture; however, the difference was not statistically significant ($P = 0.057$). This is explained by a degree of variation in the compositional data between trials, which influenced the statistical analysis of the data. The coefficients of variation of average moisture content of the experimental cheeses between 3 trials were below 5%, which is considered acceptable (Thomsson et al., 2014). Higher moisture and MNFS levels in CFHHT cheeses are partly attributed to the negative effect of HHT centrifugate on syneresis (expulsion of whey) of rennet-induced milk gels. Denatured whey protein present in HHT centrifugate can sterically hinder the aggregation (fusion) of destabilized casein micelles, as described before, and thus hinders syneresis (Pearse et al., 1985; Walstra et al., 1985; Vasbinder et al., 2003). Moreover, the high water-binding capacity of the denatured whey proteins may increase the level of MNFS in the CFHHT cheeses (Donato and Guyomarc'h, 2009). These results are in agreement with the results of Guinee et al. (1998) and Rynne et al. (2004), who observed increased moisture and MNFS of Cheddar cheese with increasing levels of denatured whey protein.

Some studies also observed a decrease in the level of total calcium in cheese made from HHT milk (Guinee et al., 1998); however, such results were not observed in the current study. The mean levels of lactose and galactose were very low, below ~67 and ~36 mg/100 g of cheese, respectively, until 11 d of ripening, and lactose and galactose were not detected after warm-room ripening (Table 2). Low lactose and galactose contents within this cheese type were expected, as the lactose contents of cheese curd were reduced by curd washing.

Age-Related Changes in pH

The pH of all experimental cheeses increased ($P < 0.001$) over the 180 d of ripening (Figure 3A, Table 3) from a mean value of ~5.2 at 1 d to ~5.7 at 180 d. This trend is in agreement with that reported in a previous study of Gouda cheese by Lawrence et al. (1987), who also observed an increase in pH from ~5.15 at 1 d to ~5.5 to 5.9 at 150 d of ripening. The increase in the pH is attributed to several factors, including the proteolytic liberation of basic compounds, such as ammonia, free basic AA, and amines (Fenelon and Guinee, 2000; McSweeney, 2004). A reduction in lactate-to-protein ratio during maturation of cheese (Figure 3B) is known to increase the buffering capacity of cheese (Sheehan

et al., 2007a), which may contribute to some extent to the increase in cheese pH during ripening. The pH of the cheese curd was significantly ($P < 0.001$) negatively correlated (adjusted $R^2 = 0.53$) with lactate-to-protein ratio (Figure 3C).

In some brine-salted cheese types, such as Gouda, Edam, and Maasdam, the pH after brining is controlled by adjusting the residual curd lactose content by techniques such as curd washing or whey dilution (Lawrence et al., 1987). More recently, membrane separation techniques, such as ultrafiltration, have also been used for standardization of lactose content of cheese milk before cheesemaking (Moynihan et al., 2016). In the present study, ~34% (wt/wt) of whey was replaced with ~23% (wt/wt) warm reverse-osmosis water to control the postmanufacture reduction of pH. In contrast, O'Sullivan et al. (2016) observed a decrease in pH at the early stages of ripening in Swiss-type cheese (where whey was not replaced by warm water); however, the postbrining pH was higher (~5.6) than that of the cheese in the current study (~5.3). The decrease in the pH at the early stages of ripening has been attributed to continual metabolism of residual lactose and galactose to lactate by starter and non-starter lactic acid bacteria (**NSLAB**; O'Sullivan et al., 2016). Shakeel-Ur-Rehman et al. (2004) also observed a decrease in the pH during ripening when Cheddar cheese was made from milk supplemented with lactose. Regulation of pH is critical for proper eye development in some eye-forming cheese types, and a reduction in pH can reduce the levels of colloidal calcium, which are considered essential for elastic texture of cheese (Lucey and Fox, 1993), and inhibit the growth of PAB (Sheehan et al., 2008). Elastic texture is important in the case of eye-forming cheese types to accommodate gas produced during warm-room ripening for smooth eye formation (Daly et al., 2010). No significant effect of treatment on the mean cheese pH during maturation was observed (Table 3).

Levels of L-, D- and Total Lactate

The mean level of L-lactate of all experimental cheeses was ~1.5% (wt/wt) until 11 d of ripening, which is most likely due to fermentation of glucose, lactose, and galactose by starter lactic acid bacteria, including *Lactococcus lactis* ssp. *cremoris*, *Lactococcus lactis* ssp. *lactis*, and *Lactobacillus helveticus*, during production of cheese (Beresford et al., 2001). However, the level of L-lactate decreased ($P < 0.001$) over ripening in all experimental cheeses, especially during warm room ripening from a mean of ~1.5% (wt/wt) at 11 d to ~0.4% (wt/wt) at 41 d (Figure 4A). This trend is in agreement with that reported in previous studies

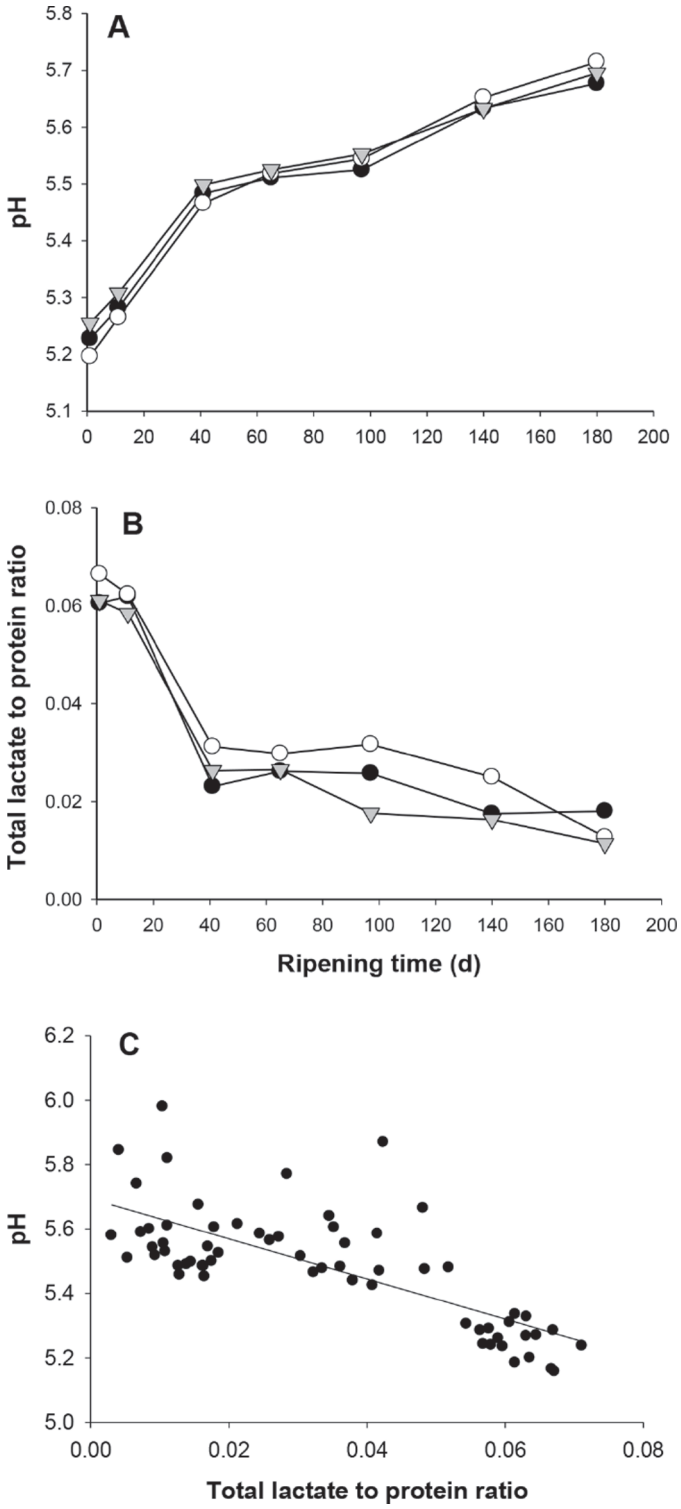

Figure 3. The effect of milk pretreatments on (A) pH and (B) lactate to protein ratio of Maasdam cheese during maturation. Milk pretreatments were control (●); centrifugation (▼); and centrifuged milk containing high-heat-treated centrifugate (○). Data presented are means of data from 3 replicate trials. (C) Relationship between pH and lactate-to-protein ratio; data were obtained from all experimental cheeses produced in 3 replicate trials and analyzed over a 180 d of ripening.

Table 3. Summary of the effects of treatment, time, and their interactions on properties of Maasdam cheeses[1]

Parameter[2]	Treatment	Time	Interactive effect (treatment × time)
pH	NS (0.81)	***	NS (1.00)
Total lactate-to-protein ratio	NS (0.33)	***	NS (0.99)
L-Lactate	NS (0.70)	***	NS (0.99)
D-Lactate	NS (0.32)	***	NS (0.99)
Total lactate	NS (0.53)	***	NS (0.99)
pH 4.6-SN (% TN)	NS (0.65)	***	NS (1.00)
Total FAA	NS (0.74)	***	NS (0.96)
Hardness	*	NS (0.29)	NS (0.97)
Springiness	NS (0.71)	*	NS (0.40)
Cohesiveness	NS (0.08)	***	NS (0.57)
Resilience	NS (1.00)	**	NS (0.96)

[1]Digits in parentheses after NS represent *P*-value.

[2]pH 4.6-SN (% TN) = soluble nitrogen at pH 4.6 as percentage of total nitrogen; FAA = free AA.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, NS = $P > 0.05$.

for different cheese varieties, such as Swiss-style cheese (Sheehan et al., 2008; O'Sullivan et al., 2016), Grevé (eye-forming, semihard cheese types; Rehn et al., 2011), and Cheddar (Rynne et al., 2007). This was expected, as L-lactate is metabolized by starter and nonstarter bacteria to different metabolites, such as propionate, acetate, butyrate, formate, succinate, DL-lactate, CO_2, H_2, and H_2O (McSweeney, 2004; Agarwal et al., 2006).

D-Lactate was virtually absent for all experimental cheeses at 1 and 11 d of ripening. However, unlike L-lactate, the level of D-lactate increased ($P < 0.001$) during warm room ripening, from a mean of ~0.01% (wt/wt) at 11 d to ~0.2 to 0.3% (wt/wt) at 41 d (Figure 4B). D-Lactate within cheese matrix typically arises either from fermentation of glucose, lactose, or galactose by microorganisms, including *Lactobacillus helveticus* and some *Leuconostoc* spp. (Beresford et al., 2001), or by racemization of L-lactate by NSLAB (Agarwal et al., 2006). As the levels of residual lactose and galactose were very low in all experimental cheeses before warm-room ripening (below 70 and 40 mg/100 g, respectively) due to curd washing, it may be assumed that the contribution of residual lactose and galactose for formation of D-lactate is minimal. The formation of D-lactate is most likely due to racemization of L-lactate to DL-lactate by NSLAB because the level of NSLAB reached ~10^8 cfu/g in all cheeses at 41 d of ripening (data not shown). Similar trends have previously been reported in different cheese varieties, such as half-fat Cheddar (Rynne et al., 2007) and Swiss-type cheese (O'Sullivan et al., 2016). Interestingly, the level of D-lactate decreased gradually after 65 d of ripening, probably due to its metabolism by microorganisms within the cheese matrix such as PAB (O'Sullivan et al., 2016). The degradation pathways of D-lactate in the cheese matrix are not yet fully understood. No significant effect of treatment was observed in the levels of L-lactate, D-lactate, and total lactate (Figure 4C) throughout ripening (Table 3).

Proteolysis

Nitrogen Soluble at pH 4.6. Primary proteolysis in cheeses was assessed by measuring the level of nitrogen soluble at pH 4.6, as a percentage of total nitrogen, which increased ($P < 0.001$) in all cheeses over the 180 d of ripening, especially during warm-room ripening, from ~5% of total nitrogen before warm-room ripening (11 d) to ~17% of total nitrogen at the end of warm-room ripening (41 d) in all experimental cheeses (Figure 5A). The levels in all cheeses reached ~22% at 180 d. The increase was of the same order of magnitude as that previously reported for semihard cheeses (Exterkate and Alting, 1995; Sheehan et al., 2007b; Huc et al., 2014). However, no significant effect of treatment on level of nitrogen soluble at pH 4.6, as a percentage of total nitrogen was observed (Table 3).

Total and Individual FAA. The mean levels of total FAA increased ($P < 0.001$) during ripening, especially when the cheeses entered the hot-room ripening phase, from ~3,000 mg/kg at 11 d to ~7,000 to 8,000 mg/kg at 41 d (Figure 5B). Enzymes from starter and nonstarter microorganisms and somatic cells, such as proteases and peptidases, contribute to primary and secondary proteolysis where present within the cheese matrix, and thereby to liberation of FAA during ripening (McSweeney, 2004; Kelly et al., 2006). No significant effect of treatment was observed (Table 3), although it was expected that the centrifugation process could alter secondary proteolysis, as the process can remove ~86 to 92% of total indigenous bacteria and ~95% of somatic cells from cheese milk (Te Giffel and Van Der

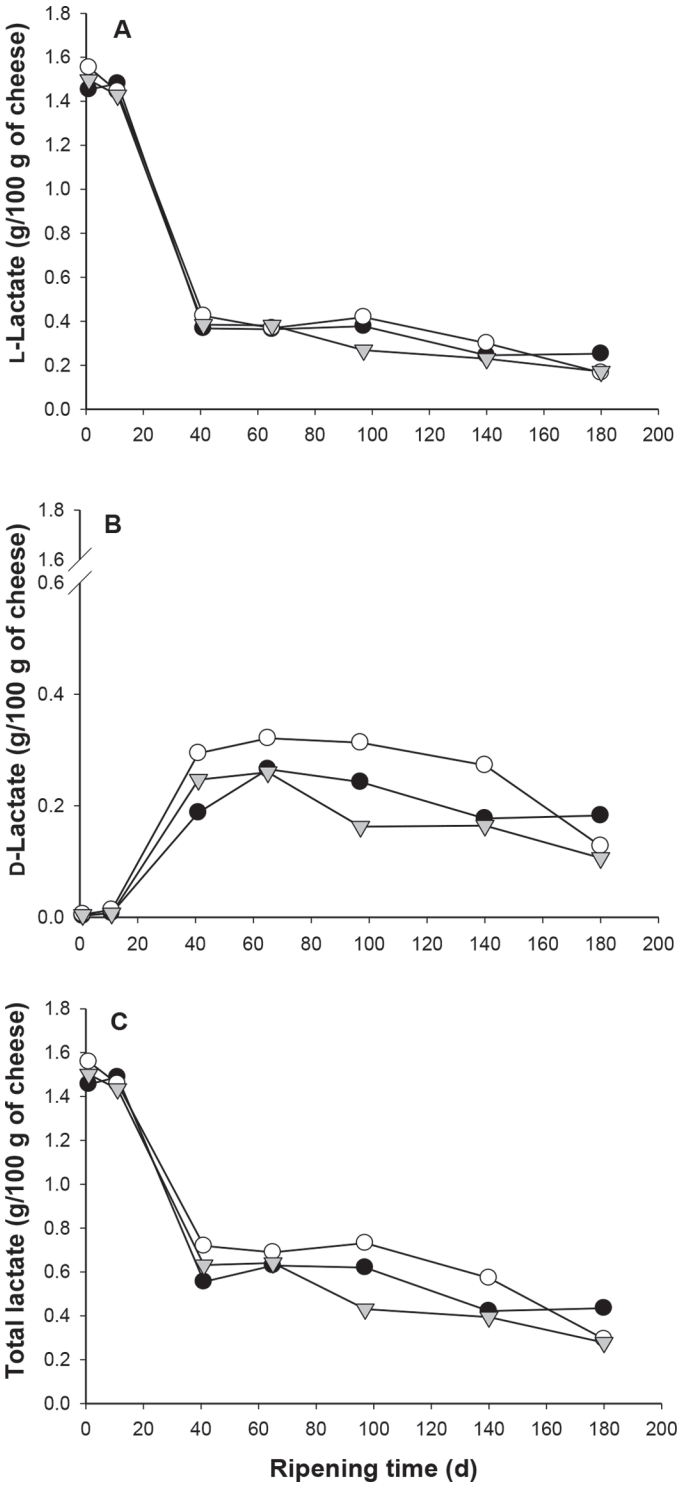

Figure 4. The effect of milk pretreatments on the mean level of (A) L-lactate, (B) D-lactate, and (C) total lactate of Maasdam cheeses during ripening. Milk pretreatments were control (●); centrifugation (▼); and centrifuged milk containing high-heat-treated centrifugate (○). Data presented are means of data from 3 replicate trials.

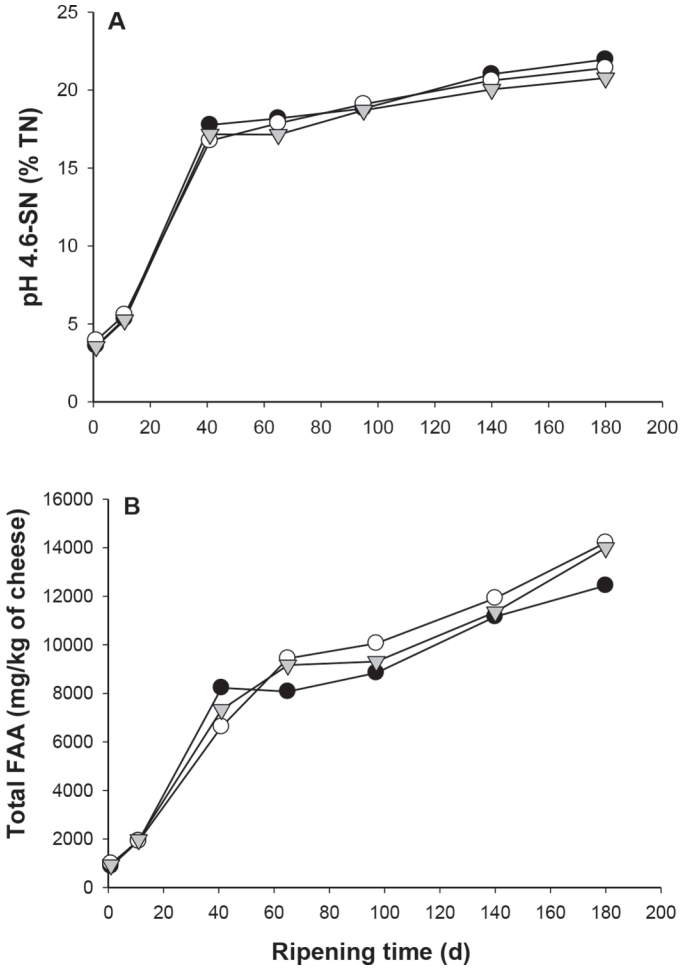

Figure 5. The effect of milk pretreatments on mean level of (A) pH 4.6-soluble nitrogen of percentage of total nitrogen (pH 4.6-SN, % TN), and (B) total free amino acids (FAA) of Maasdam cheeses during ripening. Milk pretreatments were control (●); centrifugation (▼); and centrifuged milk containing high-heat-treated centrifugate (○). Data presented are means of data from 3 replicate trials.

Horst, 2004; Wieking, 2004). In the current study, the centrifugation process also reduced the SCC by ~95%.

The concentrations of individual FAA (mg/kg) in cheeses at 140 d of ripening are shown in Figure 6. Leucine was the most abundant FAA found in all experimental cheeses, with ~2,300 mg/kg at 140 d, followed by Glu, Phe, Val, Lys, Pro, and Thr. Similar to this result, O'Sullivan et al. (2016) also observed a high level of Glu, Leu, Val, Lys, and Pro in Swiss-type cheese at 95 d of ripening. In contrast, the concentrations of Asp, Ser, Gly, Cys, Tyr, and Arg were among the lowest of the FAA. Free AA are important precursors for the formation of different classes of volatiles, such as amines, aldehydes, alcohols, acids, and sulfur compounds (Engels et al., 1997; Yvon and Rijnen, 2001).

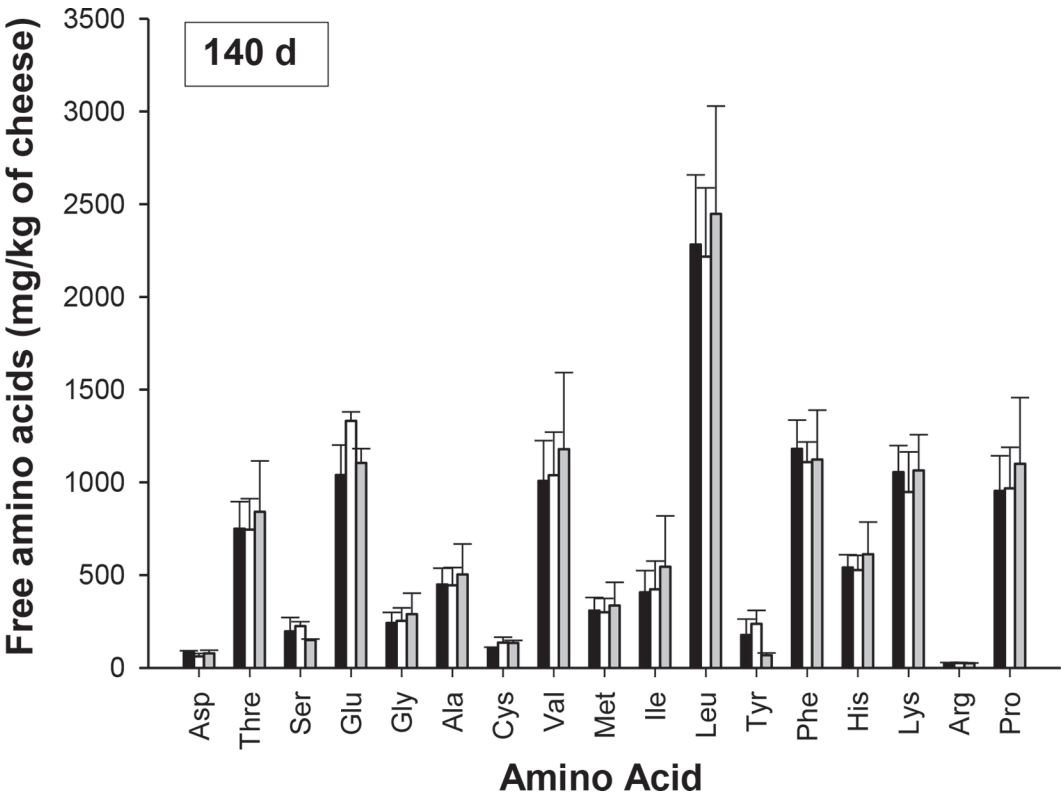

Figure 6. The effect of milk pretreatments on the mean levels of individual free AA in pH 4.6-soluble nitrogen extracts from Maasdam cheeses at 140 d of ripening. Milk pretreatments were control (black bars), centrifugation (white bars); and centrifuged milk containing high-heat-treated centrifugate (gray bars). Data presented are means of data from 3 replicate trials. Error bars show the SEM from 3 replicate trials.

No significant effect of treatment on the mean levels of individual FAA at 140 d of ripening was observed.

Texture Profile Analysis

The incorporation of HHT centrifugate into cheese milk decreased ($P < 0.05$) the mean level of instrumentally measured hardness of the resultant cheeses compared with CT and CF cheeses (Figure 7). This was attributed to significantly higher MNFS level in the CFHHT cheeses than CT and CF cheeses; MNFS is considered a good indicator of moisture associated with proteins (Lawrence et al., 1993). Moisture in the cheese matrix acts as a plasticizer between the protein strands, making cheese softer and more flexible. Moreover, during coagulation, the whey protein and whey protein-casein micelle aggregates may hinder the close approach of casein micelles during aggregation (fusion) of destabilized casein micelles; this may result in a weaker gel and curd texture (Waungana et al., 1996). From a materials science perspective, the strength of a material is known to be influenced by factors such as the extent of cross-linking and the orientation or the structural regularity of the constituents of the material (Pastorino et al., 2003; Lamichhane et al., 2018a). It may be assumed that denatured whey protein can alter the extent of cross-linking of casein micelles and the orientation or the structural regularity of casein networks within the cheese matrix.

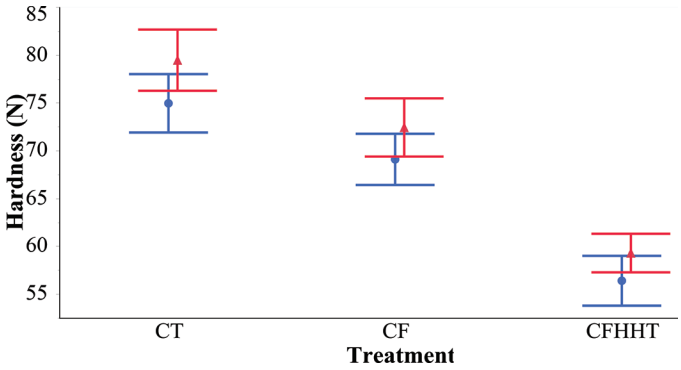

Figure 7. The effect of milk pretreatments on mean levels of hardness between 1 (●) and 11 d (▲) of ripening. Experimental cheese types were CT = control cheese, CF = cheese made from centrifuged milk, CFHHT = cheese made from centrifuged milk containing high-heat-treated centrifugate. Error bars show the SEM from 2 replicate trials. Color version available online.

No significant effects of treatment on mean levels of cohesiveness, resilience, and springiness in the cheeses were observed (Table 3); however, the values for these texture parameters decreased ($P < 0.05$) between 1 and 11 d of ripening (data not shown). Although the exact reasons for this are unknown, this may be attributed to solubilization of colloidal calcium during the early stages of ripening (O'Mahony et al., 2005). O'Mahony et al. (2005) also observed rapid decrease in the value for springiness and cohesiveness of Cheddar cheese between 1 and 21 d of ripening. Levels of insoluble calcium were not determined in cheeses in the present study, and we suggest that this should be a focus for future studies in Maasdam-type cheese. We found no significant difference in the mean level of hardness of cheese between 1 and 11 d of ripening, contrary to the results obtained by O'Mahony et al. (2005), who observed rapid decrease in the texture value within first 21 d of ripening of Cheddar cheese; this discrepancy may be attributed to different cheese types and different manufacturing steps. We were unable to analyze the texture profile of cheese after 11 d of ripening due to eye formation.

CONCLUSIONS

We demonstrated the effect of centrifugation and incorporation of HHT centrifugate on the composition, texture, and ripening characteristics of Maasdam cheese. Interestingly, centrifugation of cheese milk before cheesemaking appeared to have minimal effect on composition and age-related changes on texture, pH, proteolysis, and lactate levels of Maasdam cheese. However, incorporation of HHT centrifugate into cheese milk at levels of approximately 6 to 10% (wt/wt), depending on the protein content of centrifugate, into cheese milk significantly increased MNFS levels and also significantly decreased cheese hardness compared with control cheeses and cheeses made from centrifuged milk. Composition and strength of curd are considered important for eye-development characteristics of cheese without slits and cracks. In the current study, no clear trend for eye characteristics was observed between the treatments, and thus we were unable to draw a conclusion regarding the effect of the treatments applied on eye quality of cheese. We propose that this should be the focus of further research, possibly requiring analysis of a large number of commercial samples over the course of a manufacture season.

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