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Response surface methodology modeling of protein concentration, coagulum cut size, and set temperature on curd moisture loss kinetics during curd stirring

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ABSTRACT

The effects of the independent variables protein concentration (4–6%), coagulum cut size (6–18 mm^3), and coagulation temperature (28–36°C) on curd moisture loss during in-vat stirring were investigated using response surface methodology. Milk (14 kg) in a cheese vat was rennet coagulated, cut, and stirred as per semihard cheesemaking conditions. During stirring, the moisture content of curd samples was determined every 10 min between 5 and 115 min after cutting. The moisture loss kinetics of curds cut to 6 mm^3 followed a logarithmic trend, but the moisture loss of curds from larger cut sizes, 12 or 18 mm^3, showed a linear trend. Response surface modeling showed that curd moisture level was positively correlated with cut size and negatively correlated with milk protein level. However, coagulation temperature had a significant negative effect on curd moisture up to 45 min of stirring but not after 55 min (i.e., after cooking). It was shown that curds set at the lower temperature had a slower syneresis rate during the initial stirring compared with curds set at a higher temperature, which could be accelerated by reducing the cut size. This study shows that keeping a fixed cut size at increasing protein concentration decreased the level of curd moisture at a given time during stirring. Therefore, to obtain a uniform curd moisture content at a given stirring time at increasing protein levels, an increased coagulum cut size is required. It was also clear that breakage of the larger curd particles during initial stirring can also significantly influence the curd moisture loss kinetics. Both transmission and scanning electron micrographs of cooked curds (i.e., after 45 min of stirring) showed that the casein micelles were fused at a higher degree in curds coagulated at 36°C compared with 28°C, which confirmed that coagulation temperature causes a marked change in curd microstructure during the earlier stages of stirring. The present study showed the dynamics of curd moisture content during stirring when using protein-concentrated milk at various set temperatures and cut sizes. This provides the basis for achieving a desired curd moisture loss during cheese manufacture using protein-concentrated milk as a means of reducing the effect of seasonal variation in milk for cheesemaking.

Key words: protein standardization, cut size, coagulation temperature, curd moisture loss kinetics, electron microscopy

INTRODUCTION

Application of membrane filtration systems to concentrate milk results in retention of the larger components (i.e., protein and fat) with removal of whey as permeate. Interest in application of UF in cheesemaking has increased (Heino et al., 2010; Govindasamy-Lucey et al., 2011; Ozturk et al., 2015) because it facilitates increased cheese plant throughput and helps to achieve greater cheese consistency despite seasonal variations in milk composition (Broome et al., 1998; Guinee et al., 2006). Milk of approximately 3% protein can be concentrated to approximately 4 to 5% protein (Guinee et al., 1994; Broome et al., 1998; i.e., low concentration ratio of 1.2 to 1.6 times the normal concentration) for cheesemaking using conventional industrial cheese vats and downstream equipment (Oommen et al., 2000; Govindasamy-Lucey et al., 2005). Higher ratios add complexity to the process and require specialized equipment (Mistry and Maubois, 2017). Standardizing milk above 5% protein presents challenges during cheesemaking because of increased curd firmness and the re-

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sultant altered dynamics of rennet-induced coagulation and whey expulsion from curds (Guinee et al., 1994).

The underlying mechanism of rennet-induced gel formation in milk is related to the formation of interconnected protein networks by the action of rennet. The resulting protein matrix enmeshes large components (e.g., fat globules) by stopping their mobility (Ong et al., 2011, 2013), whereas small components (e.g., lactose and minerals) diffuse from areas of higher concentration toward areas of lower concentration. Cheesemaking includes cutting a rennet-induced gel at suitable firmness into small curd pieces using cheese knives, followed by in-vat stirring to facilitate expulsion of entrapped water and whey (Dejmek and Walstra, 2004). In modern industrial cheesemaking processes, the time allowed for stirring in-vat (vat residence time) is standardized for different cheese types.

Expulsion of whey from the curds during stirring occurs due to contraction of the protein network (Fagan et al., 2017). Curd moisture content decreases in line with the level of whey expulsion (Mateo et al., 2009a). Studies on the rate of whey expulsion during in-vat stirring suggest various orders of kinetics (e.g., first order, second order, or even higher; Caron et al., 2001; Huber et al., 2001; Thomann et al., 2006; Giroux et al., 2014), probably because of different factors that influence protein network contraction (Janhøj and Qvist, 2010).

Whey expulsion from curd is a complex process influenced by multiple factors, such as milk composition, temperature, pH, rennet level, addition of $CaCl_2$, and curd dimensions (Kaytanli et al., 1994; Thomann et al., 2006; Mateo et al., 2009a). Interactions between these factors critically affect the rate of moisture loss during in-vat stirring (Fagan et al., 2007; Giroux et al., 2014). At microscopic or macroscopic levels, moisture loss from a curd is related to breaking of protein–protein bonds, inducing microstructural changes in curds (Geng et al., 2011) and leading to the rearrangement of casein micelles in the protein network (Mellema et al., 2002). Cheesemaking from protein-standardized milk results in rapid gel firming and excessive firmness; however, the rate of whey expulsion has been reported to increase (Casiraghi et al., 1987), remain unchanged (Peri et al., 1985), or decrease (Calvo and Espinoza, 1999; Caron et al., 2001; Thomann et al., 2008) depending on the extent of milk concentration. Thus, this area would benefit from more studies, leading to greater understanding of the effects of process parameters on whey expulsion.

Temperature is an important factor that influences coagulation and whey syneresis (Castillo et al., 2006). Although temperatures for semihard cheesemaking are generally between 31 and 32°C for coagulation and 37 and 38°C for cooking, curd contraction rate increases with increasing temperature, which results in subsequent increases in whey expulsion (Huber et al., 2001; Giroux et al., 2014). Increasing protein concentration in milk further promotes rapid gel-firming rate and excessive firmness, potentially causing difficulty in curd handling during in-vat stirring (Guinee et al., 1994). Lowering of coagulation temperature has been used to normalize such coagulation properties in the case of UF-treated milk (Guinee et al., 1994; Govindasamy-Lucey et al., 2004, 2011).

Coagulum cut size is another important factor that influences whey expulsion. Curd particles of smaller size release whey faster compared with curd particles of bigger size (Grundelius et al., 2000). Coagula for hard cheese (which requires low moisture level in curds) are cut to cube sizes of 4 to 6 mm (Govindasamy-Lucey et al., 2004, 2011), whereas cube sizes for medium- to high-moisture cheeses vary between 11 and 21 mm (Renault et al., 1997; Grundelius et al., 2000). Therefore, curd size can be manipulated to adjust the rate or extent of whey expulsion (Renault et al., 1997; Johnston et al., 1998; Everard et al., 2008).

Although various methods for syneresis measurement are available (Fagan et al., 2017), measurement of curd moisture could offer more practical information for the selection of process conditions to achieve decision making for appropriate curd moisture content before drainage. Previous studies have explored the possibility of prediction of real-time curd moisture (Mateo et al., 2009a) through sensors that are attached in-line to vats to achieve better control (Mateo et al., 2009b). Modeling of interactive effects of temperature, cut size, and protein concentration through response surface methodologies can provide insights to cheesemakers for optimization, minimization, or maximization of in-vat curd moisture content during stirring. An understanding of such interactive effects on moisture loss from curd when concentrated milk is used is particularly desirable because this determines the combination of conditions that can be used to achieve consistency in curd moisture levels during stirring.

Panthi et al. (2018) showed that, under constant pH and temperature conditions, curd moisture loss after cutting follows a power law trend. It is also known that less whey expulsion occurs at lower temperatures (Castillo et al., 2006; Giroux et al., 2014). To study the practical implications of increasing or reducing the coagulation temperature, a study was designed to (1) incorporate modern cheesemaking practices such as UF concentration of milk and standardization of pH; (2) minimize initial pH change so that the model could focus on protein concentration, cut size, and set temperature; (3) use a fixed cook temperature; and (4) include a whey dilution step, such as that used for making Maasdam, Gouda, Swiss, and other cheeses, but separate from the cooking step. To achieve this, response surface methodology was used to investigate the interactive effects of protein level (4, 5, and 6%), coagulum cut size (6-, 12-, and 18-mm cubes), and coagulation temperature (28, 32, and 36°C) on curd moisture loss kinetics during in-vat stirring along with characterization of the microstructure of the curds using electron microscopy.

MATERIALS AND METHODS

Milk Collection and UF

Fresh bovine milk was obtained from the George B. Caine Dairy Research and Teaching Center (Wellsville, UT) and transported to the Gary Haight Richardson Dairy Products Laboratory at Utah State University (Logan). The whole milk was first pasteurized at 73°C for 15 s and then (~180 kg) circulated through an UF plant using spiral-wound polyethersulfone membranes with a 10-kDa molecular weight cut-off (model ST-2-3838, 10 cm × 100 cm with a 0.76-mm spacer, 7 m^2 surface area; Synder Filtration, Vacaville, CA) at approximately 45°C until approximately 3× concentration was reached. Pasteurized skim milk was purchased from a local grocery in Logan, Utah. Whole milk, UF retentates, permeates, and skim milk were stored at 4°C until needed.

Milk Standardization and Preparation

Steps for milk preparation and cheesemaking procedure are schematically presented in Figure 1. Milks were standardized targeting to nominal protein levels of 4, 5, or 6% while maintaining a protein-to-fat ratio of approximately 1.0 by combining pasteurized whole milk, skim milk, and UF retentate in 14-kg rectangular cheese vats. The protein-to-fat ratio was chosen to target a semihard-type cheese manufacturing process for production of cheese with 45% fat in DM. Standardized milk pH varied between 6.72 and 6.77, and this was adjusted to 6.5 by adding lactic acid (1:8 dilution, vol/vol). Milk was then heated to 45°C and cooled to the set temperature to minimize the effect of cold storage (Qvist, 1979).

Milk Coagulation

Milks were adjusted to the required set temperature (28, 32, or 36°C) and inoculated (20 g/kg of milk) with frozen pellets of *Lactococcus lactis* ssp. *lactis/cremoris* (DVS850, Chr. Hansen Inc., Milwaukee, WI). The starter culture was added approximately 10 min before adding rennet to minimize any pH decrease in the cheese vat before draining of whey, exploiting the long lag phase before exponential growth of frozen cultures (the normal ripening time allowed for frozen direct-to-the-vat cultures is 30–45 min). Double-strength (~650 international milk-clotting units/mL) chymosin (Maxiren; DSM Food Specialties USA Inc., Eagleville, PA) was added at a rate of 57 μL/kg of milk. The rennet solution (0.80 mL) was diluted in 17 mL of water before addition, giving 37 international milk-clotting units/kg of milk. For determining the time suitable for cutting of the coagulum, renneted milk was stirred for 1 min and an aliquot (~30 mL) was removed for monitoring gel formation using rheometry, as described by Panthi et al. (2019).

Curd Manufacture and Sampling

The coagulated milk was cut based on the data from a rheometer when the storage modulus (G′) reached approximately 35 Pa and was confirmed by visual observation. Cutting knives were fabricated to achieve curd cubes of 6, 12, or 18 mm (hereafter, “cube” is not mentioned before size). Coagula were cut in 3 dimensions within 1 min, healed for approximately 3 min, and gently stirred manually from 4 min after the start of cutting. For curds prepared from milks of 5 and 6% protein with 12- and 18-mm cut size, a total of 4 kg of UF permeate produced from the original milk was overlaid on the top of coagula before and after cutting to avoid curd damage during stirring. The temperature of the permeate was increased to the coagulation temperature before overlaying.

Starting from 5 min after cutting, curds were sampled every 10 min for moisture content. At 25 min after cutting, the curd and whey were heated to 37°C over 20 min, and this temperature was maintained and stirring continued. This gave a temperature increase of 9, 4, or 1°C for curds set at 28, 32, or 36°C, respectively. At 75 min after cutting, 3 kg of whey was removed, 2 kg of water at 37°C was added to dilute the lactose content of whey, and curd stirring was continued until 115 min after cutting.

Scanning and Transmission Electron Microscopy

To investigate differences in curd microstructure, samples of curd were obtained from milk containing 4 and 6% protein and set at 28 or 36°C from coagula cut into 6-mm curd pieces. Curd samples were removed at 45 min after cutting and fixed in glutaraldehyde–

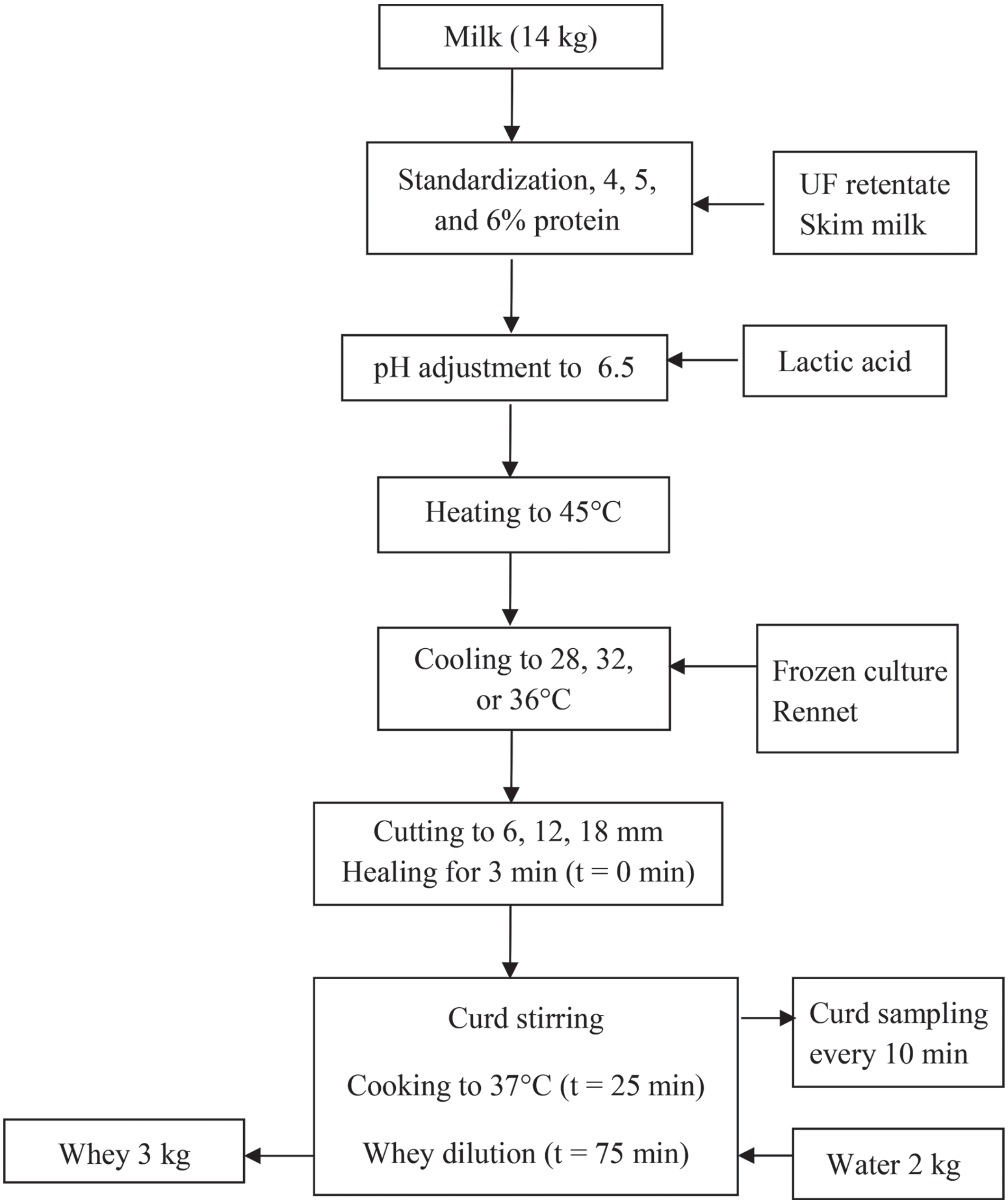

Figure 1. Schematic presentation of milk preparation and curd making.

formaldehyde for curd microstructure analysis using scanning electron microscopy and transmission electron microscopy as described by Panthi et al. (2019).

Compositional Analysis

Composition of milk, permeate, retentate, and skim milk was determined with a Fourier-transform infrared spectroscopy milk analyzer (Bentley Instruments Inc., Chaska, MN). Mineral contents of milk were determined using inductively coupled plasma optical emission spectrometry (Thermo iCAP 6300, Thermo Fisher Scientific, Waltham, MA) after wet digestion as described by Gavlak et al. (2005). Curd moisture contents were determined in triplicate using a hot air oven method as described by Mateo et al. (2009a).

Relative curd moisture (**RCM**) content was calculated as described by Panthi et al. (2018), and RCM data were fitted to the following empirical equation:

$$\mathrm{RCM} = \mathrm{RCM}_o - \frac{\left(\mathrm{time}^k + a\right)}{k}, \qquad [1]$$

where RCM_o is relative curd moisture content at time 0 = 100, k is the kinetic constant, and a is another constant factor in the model. Values of k, a, and the coefficient of determination (R^2) were calculated.

Statistical Analysis and Experimental Design

Milk composition was analyzed using 1-way ANOVA with Tukey honest significance difference for comparing means using SPSS Statistics (version 24; IBM, Armonk, NY) at a 95% significance level. Curd moisture loss kinetics data obtained from Equation 1 were analyzed using nonlinear least squares (nls) function and plotted using the ggplot2 package in the R studio (version. 1.1.383) of the R program (version 3.4.2, R Foundation for Statistical Computing, Vienna, Austria).

Influence of protein concentration, set temperature, and curd size was analyzed using response surface methodology with a central composite design ($\alpha = 1$) as shown in Table 1. Temperature and protein levels were randomized, and batches with similar cut size were prepared on each day for consecutive runs. Initial trials were carried out to examine the process variation within trials (data were excluded from analysis), and then 21 cheesemaking trials were conducted for data analysis. The full polynomial model (Equation 2) was used to determine the relationship between 3 variables on curd moisture:

$$Y = b_o + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{3} b_{ij} X_i X_j, \qquad [2]$$

where Y is the response variable, b_o is a model intercept, and X_i and X_j are the factor levels. The model comprises linear (b_i), quadratic (b_{ii}), and cross-product (b_{ij}) terms. Response surface modeling was carried out using PROC RSREG in SAS/STAT (version 14.3; SAS Institute Inc., Cary, NC). Cross-product terms were removed from the full model for model parameter estimate. The same reduced model was used to create contour plots using PROC REG and PROC TEMPLATE in SAS and surface plots using Design Expert (version 10, Stat-Ease Inc., Minneapolis, MN).

RESULTS AND DISCUSSION

Composition of Milk

The standardized milk compositions before cheesemaking are shown in Table 2. As expected, protein, fat, and TS contents increased significantly ($P < 0.05$) with increased protein concentration in the standardized milk, with a uniform protein-to-fat ratio (Table 2). Similarly, pH and lactose levels were not significantly affected. The levels of total calcium and phosphate increased significantly with increasing protein levels. Sandra et al. (2011) also reported a significant increase of total calcium in protein concentrated milk. Because one-third of calcium in milk is contained in the serum phase, a portion of this soluble calcium passes into permeate during UF. Hence, the calcium-to-protein ratio decreased with increasing protein content.

Curd Breakage During Stirring

There was a significant interactive effect of set temperature and protein level ($P = 0.015$; Supplemental Table S1; https://doi.org/10.3168/jds.2018-15051) on the time from renneting of milk to when the coagula was cut. Set to cut time was shorter with higher set temperature and protein levels. Thus, cutting was performed at a storage modulus of approximately 35 Pa rather than at a specified time to obtain uniform curd rigidity.

During initial stirring after cutting, breakage of curds occurred due to resistance of the curd movement, when permeate was not overlaid. Breakage of curds can be

Table 1. Variable factors and conditions used in the response surface design

	Symbol		Level		
Condition	Code	Unit	−1	0	+1
Temperature	X1	°C	28	32	36
Cut size	X2	mm^3	6	12	18
Protein	X3	% (g/100 g)	4	5	6

Table 2. Composition of standardized milk before cheesemaking

Component	Milk composition 4% Protein (n = 6)	5% Protein (n = 7)	6% Protein (n = 8)
Protein (g/100 g)	3.94^{c}	5.03^{b}	6.02^{a}
Fat (g/100 g)	3.95^{c}	5.04^{b}	6.05^{a}
Protein-to-fat ratio	1.00^{a}	1.00^{a}	0.99^{a}
Lactose (g/100 g)	4.76^{a}	4.60^{a}	5.02^{a}
TS (g/100 g)	13.52^{c}	15.66^{b}	17.43^{a}
Total calcium (g/kg)	1.33^{c}	1.60^{b}	1.93^{a}
Total phosphate (g/kg)	2.96^{c}	3.60^{b}	4.24^{a}
Ca/protein (mg/g)	33.6^{a}	31.7^{b}	32.05^{b}
pH	6.75^{a}	6.74^{a}	6.76^{a}

[a–c]Means within a row with the same superscript were not different (α = 0.05).

illustrated by contour plots of curd moisture content over stirring (Supplemental Figure S1A–D; https://doi.org/10.3168/jds.2018-15051). After 5 min of stirring, when the cut size used was increased from 6 to 12 mm, curds were observed to contain a greater moisture for all 4, 5, or 6% protein curds, whereas such a trend in curd moisture content was not observed when using the cut size from 12 to 18 mm, indicating breakage of curd at the large cut size. At 25 min of stirring, the effect of changing cut size was evident for curds of 4% protein as indicated by a greater curd moisture content with higher cut size from 6 to 18 mm. However, applying the same change in cut size with curds of 6% protein resulted in similar moisture contents (~72%). As the stirring continued to 45 to 115 min, the effect of changing cut size was evident for 4% protein milk, whereas the moisture content of curds with 5% protein was observed to be less influenced when using cut size from 12 to 18 mm, and for 6% protein the effect of changing cut size on curd moisture was negligible. Therefore, curds with 12- or 18-mm cut size and 5 or 6% protein were highly affected by the breakage of curds.

Curds require whey to float during stirring so that curd can move along with the motion of stirrer. However, slow release of whey from the curds from 5 or 6% protein with cut at a large size did not allow curd to move during initial stirring, which subsequently led to the breakage of curds into small particles. Maintaining the curd size during stirring was considered important in this study to establish the relationship between cut size and other variables. To avoid breakage of curds, a layer of UF permeate was overlaid on the coagula before and after cutting (2 kg + 2 kg) for trials conducted with milk with 5 and 6% protein and using a cut size of 12 and 18 mm. Overlaying permeate allowed the movement of curds during initial stirring and increased curd interparticle distance, which resulted in the curds maintaining their shape and size. As expected, permeate-overlaid curds had a higher moisture content compared with those prepared without overlay because of the retention in size (Supplemental Figure S2; https://doi.org/10.3168/jds.2018-15051). Hence, overlaying of permeate during cutting helped in establishing the relationship of cut size with other variables.

Curd Moisture Loss During Stirring

Curd moisture contents during stirring from 5 to 115 min decreased under each experimental condition (Figure 2). Interestingly, the decrease in moisture content in curds of 6-mm cut size showed a logarithmic trend, whereas the decrease in curds of 12- or 18-mm cut size was linear. Evidently, the profile of moisture loss from curds set at 28°C with cut size 6 mm was slightly different from logarithmic due to a faster decrease in curd moisture content during cooking (25–45 min of stirring). Such a rapid decrease in moisture content loss was due to greater increase in temperature during cooking. However, a fast decrease in moisture even for curds set at 28°C and cut at larger cut size (12 or 18 mm) was less evident. Probably, the contraction of the curds occurs at the curd surface and the presence of a larger proportion of moisture in the internal curd matrix minimizes the magnitude of moisture loss by temperature increase. Interestingly, the rate of moisture loss after 45 min of stirring showed a linear trend in each condition.

It is apparent that curd from milk with a higher protein concentration attained lower moisture content at a given time compared with the curd from milk with a lower protein content. However, the rate of moisture loss was not influenced by protein concentration, as indicated by a similar slope of the moisture loss curve at similar conditions of set temperature and cut size (Figure 2). This finding is consistent with the results of Peri et al. (1985), who reported that expulsion of whey from curd was independent of protein concentration in milk, which was investigated within approximately 5× protein concentration of milk. The results from the present study also agree with the findings of Thomann et al. (2008), who observed greater volume of total liquid collected when combining permeate release during membrane filtration and expelled whey during curd stirring from concentrated curd compared with total whey expelled from regular milk.

Prediction of Curd Moisture

Prediction of in-vat curd moisture content using an empirical model is currently of interest (Panthi et al., 2018). The present study demonstrates various profiles of curd moisture loss depending on curd size and set temperature, emphasizing the requirement of a model that can explain the curd moisture loss under variable conditions. The RCM data were fitted to Equation 1; however, this poorly explained the curd moisture data from the curd cut at 18 mm at 28°C or 32°C (R^2 = 0.79–0.88). There was a better fit with data from curds set at 36°C (R^2 = 0.90), probably due to there being only a 1°C increase during cooking, resulting in an almost constant temperature during stirring. A representative graph of the model fit on RCM data is presented in Figure 3. The model explained the moisture loss profile of curds at small and large cut sizes, with R^2 values being 0.98 and 0.90, respectively. As expected, curds coagulated at 36°C with 4% protein and cut at 6 mm had faster moisture loss kinetics (k = 0.51) compared with those cut at 18 mm (k = 0.30). Curds of small particle size have a larger surface area and a shorter distance from center to edge for whey movement compared with bigger curd particles, which have a much smaller surface-to-volume ratio, resulting in restricted whey expulsion and subsequent greater retention of moisture (Renault et al., 1997; Dejmek and Walstra, 2004).

In this study, curd size was varied based on spacing between the curd knives. Alternatively, curd size can be varied depending on the number of rotations of the

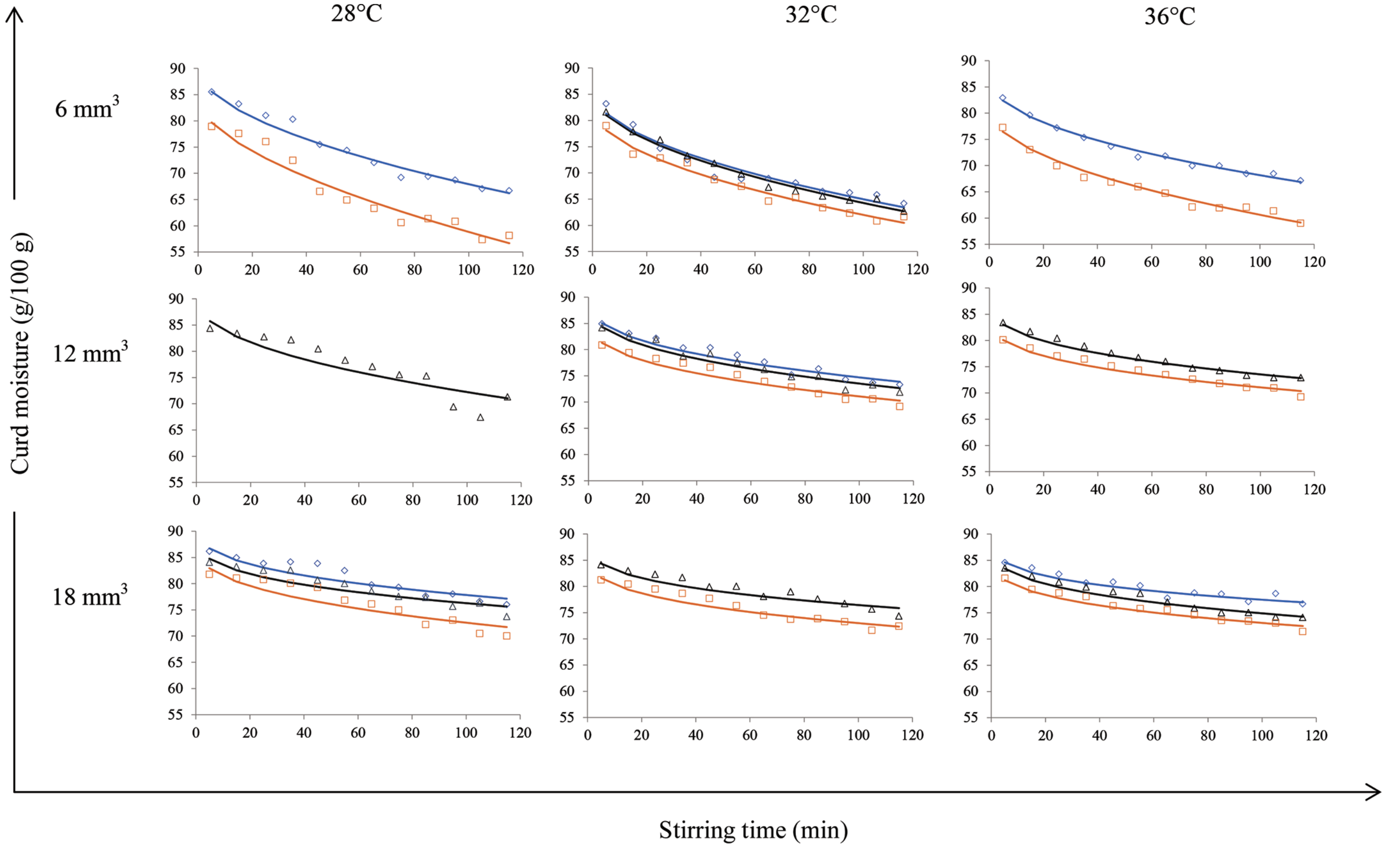

Figure 2. Moisture loss profile of curds (g/100 g) during stirring from 5 to 115 min after cutting at various conditions of set temperature (28–36°C), cut size (6–18 mm^3), and protein concentration (◊ = 4%, Δ = 5%, □ = 6%). Trend lines were drawn using Equation 1 for actual curd moisture content. Permeate was overlaid during cutting for coagulum of 5 and 6% protein with 12- and 18-mm cut size.

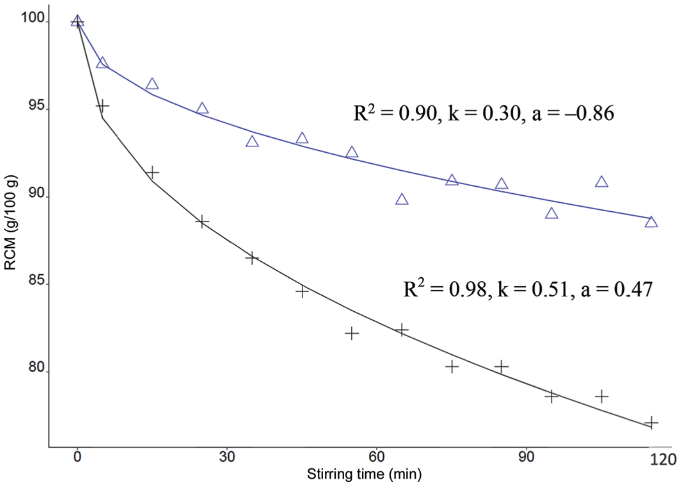

Figure 3. Representative plot of model fitted to the relative curd moisture (RCM) data as a function of time during stirring. + = 36°C, 6 mm, 4% protein; Δ = 36°C, 18 mm, and 4% protein. Coefficient of determination (R^2) and model parameters constants (k and a) are the values calculated using the model. The line indicates model prediction.

blades through the coagulum during cutting; knives are of fixed dimensions, which results in a wide range of curd size (Johnston et al., 1998; Everard et al., 2008; Mateo et al., 2009b). Although actual whey expulsion rates may vary based on the method of cutting (especially when curd particles are cut into irregular shapes), in general the same principles apply in that overall moisture loss from the curd will be influenced by the size range of the curd particles.

Response Surface Modeling of Curd Moisture During Curd Stirring

The ANOVA of the regression model fit showed that most variations in curd moisture were explained by the linear and quadratic regression terms of the model, whereas cross-product terms were nonsignificant (Table 3). To estimate the regression coefficients of the polynomial regression model, a response surface model without cross-product terms was used so that estimates could be more robust and more precise. Table 4 summarizes estimates and goodness of model fit (R^2 and coefficient of variation) of the reduced model, which was satisfactory for prediction of curd moisture at a given stirring time.

Coagulation temperature (X1) had a significant negative effect on curd moisture for the first 45 min, but not after the curd temperature reached 37°C (i.e., by the end of cooking; with the exception of 105 min). Cut size (X2) had a significant positive effect on curd moisture at all times, and this effect increased over stirring time, as indicated by increasing values of the coefficient for cut size (Table 4). The negative effect of protein concentration (X3) on curd moisture content was fairly uniform during stirring, indicating that protein concentration did not markedly influence the curd moisture loss. The significant quadratic effect of cut size (X2) indicates a nonlinear correlation between cut size and curd moisture content.

Curd Moisture Loss During Cutting and Cooking

The effects of set temperature, cut size, and protein content on the relative rate of initial curd moisture loss (during the first 5 min after cutting) compared with the phase of increasing temperature (25–min after cutting) are shown in Figure 4. The contour plots show that the rate of moisture loss follows different patterns depending on whether it is measured during the initial stirring or during cooking. As expected, the initial rate of moisture loss was proportional to the coagulation temperature and the rate of moisture loss during cooking was proportional to the temperature gradient (Figure 4A, B). In both phases, the rate of moisture loss was inversely correlated with curd cut size. The contour plot shows that when setting milk at lower temperatures, coagulum should be cut to smaller sizes to achieve similar rates of initial moisture loss than under conditions where milk is set at normal temperatures (Figure 4A). Curd cut at larger cut size (12–18 mm) had almost negligible moisture loss during initial stirring. The slow release of moisture in particles of larger cut size during initial stirring causes breakage because the expulsion of whey allows curd to move and float during stirring. The slow release of whey from a curd at lower temperature can be compensated by reducing the coagulum cut size.

On the other hand, for curds set at lower temperature (especially with smaller cut size), the rate of moisture loss during cooking was 0.32%/min and then decreased with increasing temperature or cut size to 0.07%/min for larger curds set at higher temperature. This occurred because the increase in temperature during cooking to 37°C was larger when the set temperature was 28°C compared with a set temperature of 36°C. At higher temperature, greater rearrangement of casein micelles in the protein network occurs, creating a force for curd contraction (Mellema et al., 2002). Subjecting the curd to a temperature increase enhances contraction of the protein network, which causes concomitant whey expulsion. This perhaps minimized the effect of set temperatures on curd moisture content after cooking (Table 3). However, Fagan et al. (2007) observed a significant effect of coagulation temperature on curd

Table 3. *F*-values (associated *P*-values in parentheses) for significance of polynomial model terms[1] on curd moisture throughout 115 min of curd stirring

	Curd stirring time (min)											
Model terms	5	15	25	35	45	55	65	75	85	95	105	115
Linear	165.97 (<0.0001)	125.75 (<0.0001)	61.54 (<0.0001)	51.10 (<0.0001)	56.18 (<0.0001)	98.53 (<0.0001)	213.73 (<0.0001)	86.68 (<0.0001)	106.84 (<0.0001)	123.37 (<0.0001)	67.40 (<0.0001)	127.22 (<0.0001)
Quadratic	10.24 (0.0016)	14.09 (0.0004)	9.04 (0.0026)	6.49 (0.0087)	7.49 (0.0043)	11.12 (0.0012)	30.27 (0.0001)	7.94 (0.0043)	13.48 (0.0005)	4.26 (0.0318)	3.28 (0.062)	11.43 (0.001)
Cross-product	5.94 (0.01)	5.60 (0.01)	2.51 (0.11)	1.08 (0.39)	0.41 (0.75)	0.88 (0.48)	5.85 (0.01)	0.85 (0.49)	0.37 (0.77)	1.09 (0.39)	0.75 (0.54)	0.34 (0.8)

[1] Based on coded variable of each parameter (−1, 0, +1).

Table 4. Estimates of selected model parameters[1] of set temperature (X1), cut size (X2), and protein concentration (X3) on curd moisture content during stirring

	Curd stirring time (min)											
Curd moisture[1]	5	15	25	35	45	55	65	75	85	95	105	115
Intercept	83.77	82.02	81.18	79.91	78.88	77.63	76.13	75.21	74.87	72.17	72.20	71.94
X1	−0.64*	−1.18*	−1.48*	−1.74*	−1.02*	−0.64	−0.33	−0.03	0.04	0.39	1.22*	0.46
X2	1.16*	2.26*	2.93*	3.65*	4.75*	4.97*	4.88*	5.49*	5.24*	5.46*	5.66*	5.56*
X3	−2.32*	−2.38*	−2.02*	−2.06*	−2.40*	−2.49*	−2.47*	−2.50*	−2.77*	−2.48*	−3.01*	−2.78*
X1.X1	0.03	0.66	0.61	0.81	0.35	0.19	0.57	−0.34	−0.07	−0.20	−0.67	−0.21
X2.X2	−0.87*	−1.89*	−2.36*	−2.70*	−3.58*	−3.21*	−3.66*	−3.00*	−3.56*	−1.99*	−2.29*	−3.22*
X3.X3	−0.63	−0.60	−0.94	−0.76	−0.49	−0.67	−0.44	−0.65	−0.68	0.19	−0.18	−0.29
R^2	0.98	0.93	0.91	0.92	0.94	0.96	0.96	0.95	0.97	0.96	0.94	0.97
CV	0.54	1.18	1.56	1.77	1.91	1.60	1.57	1.89	1.59	1.64	2.33	1.57

[1] Predicted model parameters based on coded variables of each parameter (−1, 0, +1).

*Significant at $\alpha = 0.05$.

moisture content during stirring (5–65 min) when cooking of curds was not undertaken.

In the present study, the effect of pH change was minimized by adding lactic acid and frozen culture and subsequently giving a shorter ripening time (~10 min) than in standard cheesemaking practice (~45 min). The pH value of whey samples measured after 75 min of stirring was not significantly influenced ($P = 0.426$) by protein concentration; however, set temperature did significantly influence pH ($P = 0.031$). The average pH of whey samples measured after 75 min was 6.31, 6.34, and 6.36 from the curds coagulated at 28, 32, and 36°C, respectively. Slight differences in the pH at 75 min suggest that pH differences during the early stages of stirring, for example at 45 min, could be negligible. It is suggested that little pH change is observed in the early stages of industrial-scale cheesemaking with the growing use of direct-vat inoculum cultures, as was observed in the present study. However, changes in pH in the early stages may be more evident when bulk cultures are used, promoting earlier curd syneresis.

Curd Moisture Content at 75 min

Curd moisture contents at 75 min as influenced by cut size and protein concentration are presented in Figure 5A. It is evident that on increasing protein concentration of milk, moisture content of the curds decreased and curd moisture content increased with increasing coagulum cut size. Similar trends in the contour plots were observed at each sampling point with reduced curd moisture contents over time (plots not shown).

Whey drainage occurs at approximately 75 min after cutting for many semihard cheese manufacturing processes. It is apparent that, at 75 min, cheese curds produced with a 6-mm cut size and from milk of 6% protein attained a low curd moisture content (60 g/100 g of curds), whereas curds of 4% protein and with an 18-mm cut size retained significantly more moisture (79 g/100 g of curds). These results demonstrate that in-vat curd moisture content varies widely with respect to cut size and protein level in milk. It is proposed that these 2 factors can be exploited to control the moisture content of curds during stirring. For example, curds with 72% moisture can be produced with milk concentrated to 4% protein with a cut size of less than 7 mm, whereas at a milk protein concentration of 6%, a cut size of approximately 12 mm is required to obtain a similar moisture level. Therefore, manufacturing cheese from milk with increased protein concentrations requires increased curd sizes to achieve similar moisture to that in standard cheese manufacturing practices. Thus, cheesemaking from milk with increased protein concentration requires modified standard operating procedures to maintain an appropriate stirring time between cutting of the curd and curd drainage.

Achieving uniform curd moisture content before drainage of curds is a key control point for consistency in the cheesemaking process. To explore curd moisture content over stirring, response surface contour plots were generated with stirring time as a factor. For example, when cut size is fixed to 12 mm at a set temperature 32°C, it will take 95 min to attain 74% moisture for curds from 4% protein milk, which is re-

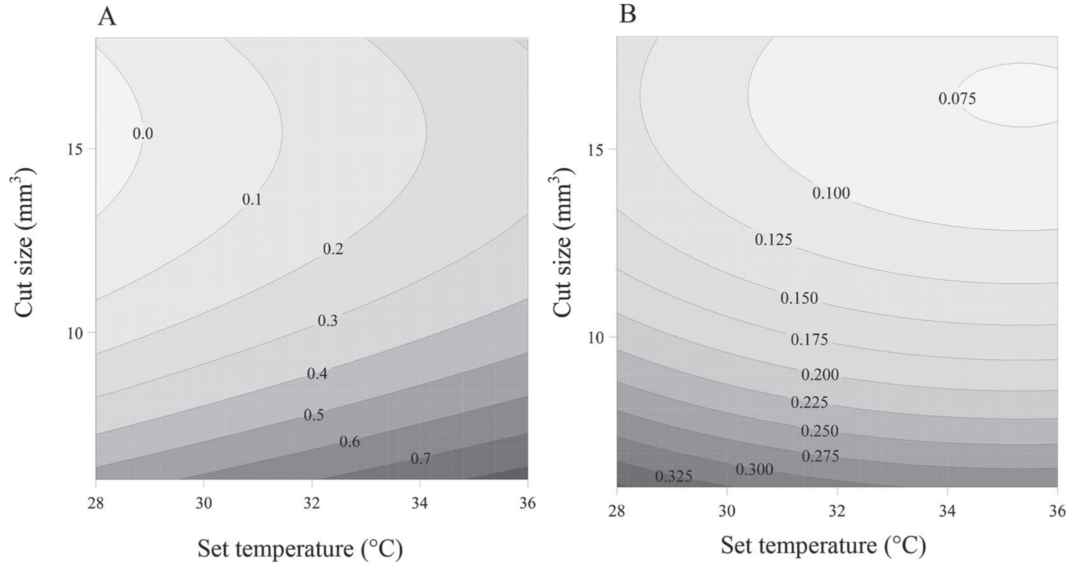

Figure 4. Contour plots of relative curd moisture loss (%/min) (A) after initial stirring and (B) during cooking as affected by set temperature and cut size. Permeate was overlaid during cutting for coagula of 5 and 6% protein with 12- and 18-mm cut size.

duced to 75 min for curds from 5% protein milk and 45 min for curds from 6% protein milk (Figure 5C). Likewise, curds with 70% moisture can be produced in 55 min when cutting a coagulum with 6-mm cut size, and more than 115 min is required to achieve a similar level of curd moisture when cut size is increased to 12 mm (Figure 5D). Therefore, concentrating milk protein levels allows processing of increased milk volume and a shorter processing time, thus increasing efficiency in 2 ways.

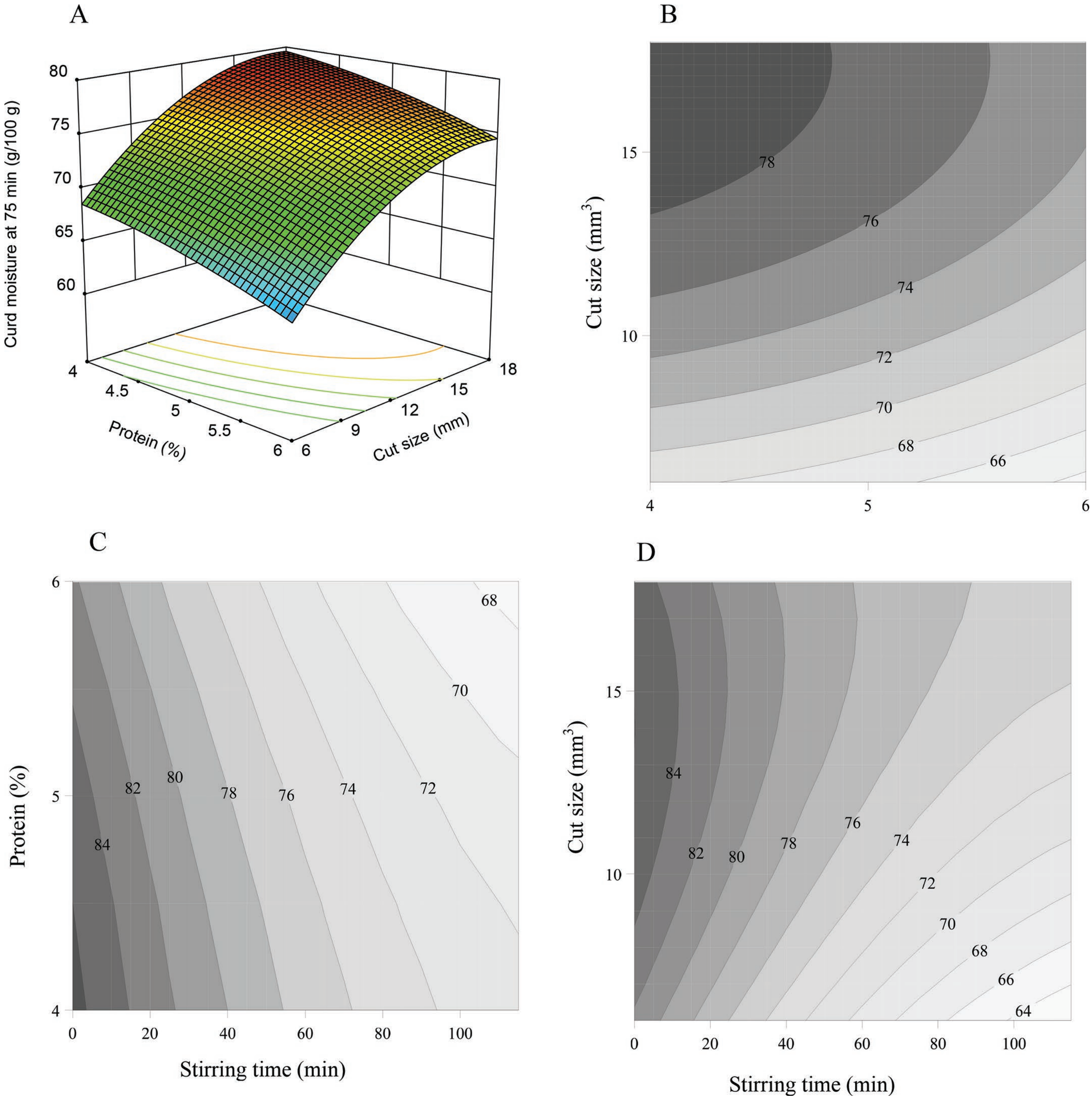

Figure 5. (A) Surface plots and (B) contour plot of curd moisture content (g/100 g) as affected by protein level and cut size at 75 min of stirring. Contour plot of curd moisture content as a function of stirring time as influenced by (C) protein level and (D) cut sizes. Permeate was overlaid during cutting for coagula of 5 and 6% protein with 12- and 18-mm cut size.

Previous studies have shown that cheese moisture content decreases when increasing protein levels to approximately 4.5% (Guinee et al., 1996, 2006; Broome et al., 1998), which could be because of lower curd moisture content before drainage. The negative correlation of moisture content with protein level can be explained partly by an increased level of protein and fat in standardized milk due to the loss of serum phase by concentration (Thomann et al., 2008). Furthermore, increasing the volume fraction of the curd particles with respect to the serum phase leads to a subsequent increase in the frequency of collisions between curd particles, contributing to greater moisture loss during stirring due to particle deformation (Guinee et al., 2006; Geng et al., 2011). Permeate overlay in the present study was expected to minimize such influences.

Some studies have suggested a decrease in syneresis rate (Calvo and Espinoza, 1999; Caron et al., 2001; Thomann et al., 2008) with increasing milk concentration ratio, whereas other studies that expressed syneresis in terms of moisture retention reported increased syneresis rate with increasing protein levels in milk (Casiraghi et al., 1987). The results of the present study confirmed that curd moisture loss in-vat was independent of protein concentration of milk and that the observed differences in curd moisture content were mainly attributed to differences in milk solid contents. This probably results in curds from concentrated milk expelling a lower weight of whey compared with those prepared from regular milk, as moisture constitutes a smaller proportion of weight of curds from concentrated milks compared with those from unconcentrated milk at any given time during stirring (Casiraghi et al., 1987; Thomann et al., 2008).

Curd Microstructure

Scanning Electron Microscopy. Curd microstructure, as influenced by coagulation temperature, is shown in Figure 6 for milk with 6% protein concentration and set at either 28 or 36°C. At low magnification, curd particles at both temperatures appeared to have a loose structure with many larger voids (Figure 6A and B). Differences in the microstructure of curds were more apparent at higher magnification, with the casein particles showing a higher degree of fusion in curds set at 36°C (Figure 6F), which resulted in slightly thicker strands and a flattened shape compared with the curd formed at 28°C (Figure 6E). Similar differences were observed at the time of cutting the coagulum (Panthi et al., 2019), but casein micelles were fused to a greater extent in the cooked curds in both treatments. For milk containing 3.3% protein, Ong et al. (2011) also observed a fine regular protein network in curds that had been set at 27°C and subsequently cooked to 38°C compared with curds set at 36°C and cooked to 38°C; the latter conditions resulted in a dense protein network with larger casein micelles aggregates.

Transmission Electron Microscopy. Figure 7 shows transmission electron micrographs of curds from different treatments. At low magnification it could be seen that casein micelles formed a regular network with fat globules interspersed in the curds when set at 28°C (Figure 7A) or 36°C (Figure 7B), but the network appeared greatly condensed with more connection points at 36°C. Medium- and high-magnification images showed that casein micelles were fused to thicker branches in the curds set at 36°C (Figure 7D and F) compared with those set at 28°C (Figure 7C and E). This correlates well with the scanning electron microscopy findings. Changes in the curd microstructure during cooking were greater for curds set at 28°C compared with 36°C. Curds set at 28°C had a loose and compact structure of casein micelles aggregates before cooking (Panthi et al., 2019), which, after cooking, appeared to have a porous structure with extensively fused casein micelles in an aggregate. Ong et al. (2013) reported no difference in the microstructure of cooked curds prepared from milk with increasing protein levels (4–5.8%) coagulated at 33°C. Thus, it appears that the temperature at which coagulation occurs has a greater influence on internal structure than protein concentration.

It seemed that curds set at 28°C probably had continuous casein micelle rearrangement during cooking because of less cross-linking of casein micelle aggregates (Figure 6E), whereas in curds set at 36°C, such phenomena probably occurred before cooking as observed by flattened and fused casein micelle aggregates (Figure 6F). Void spaces (probably filled with whey) observed in Figure 6A and B with curds set at 28 or 36°C are probably due to rearrangement of casein micelles in the protein network (containing whey and fat globules before scanning electron microscopy sample preparation) that exerted more pressure for whey, leading to accumulation of whey between casein clusters (Van Vliet et al., 1991). For curds set at 28°C, larger void spaces were observed after cooking compared with at the time of cutting (Panthi et al., 2019), indicating that protein network rearrangement was greater during cooking for curds set at 28°C compared with curds set at 36°C. This probably explains the greater moisture loss during cooking from curds set at 28°C compared with those set at 36°C.

CONCLUSIONS

Interactive effects of milk protein concentration, cut size, and coagulation temperature on curd moisture

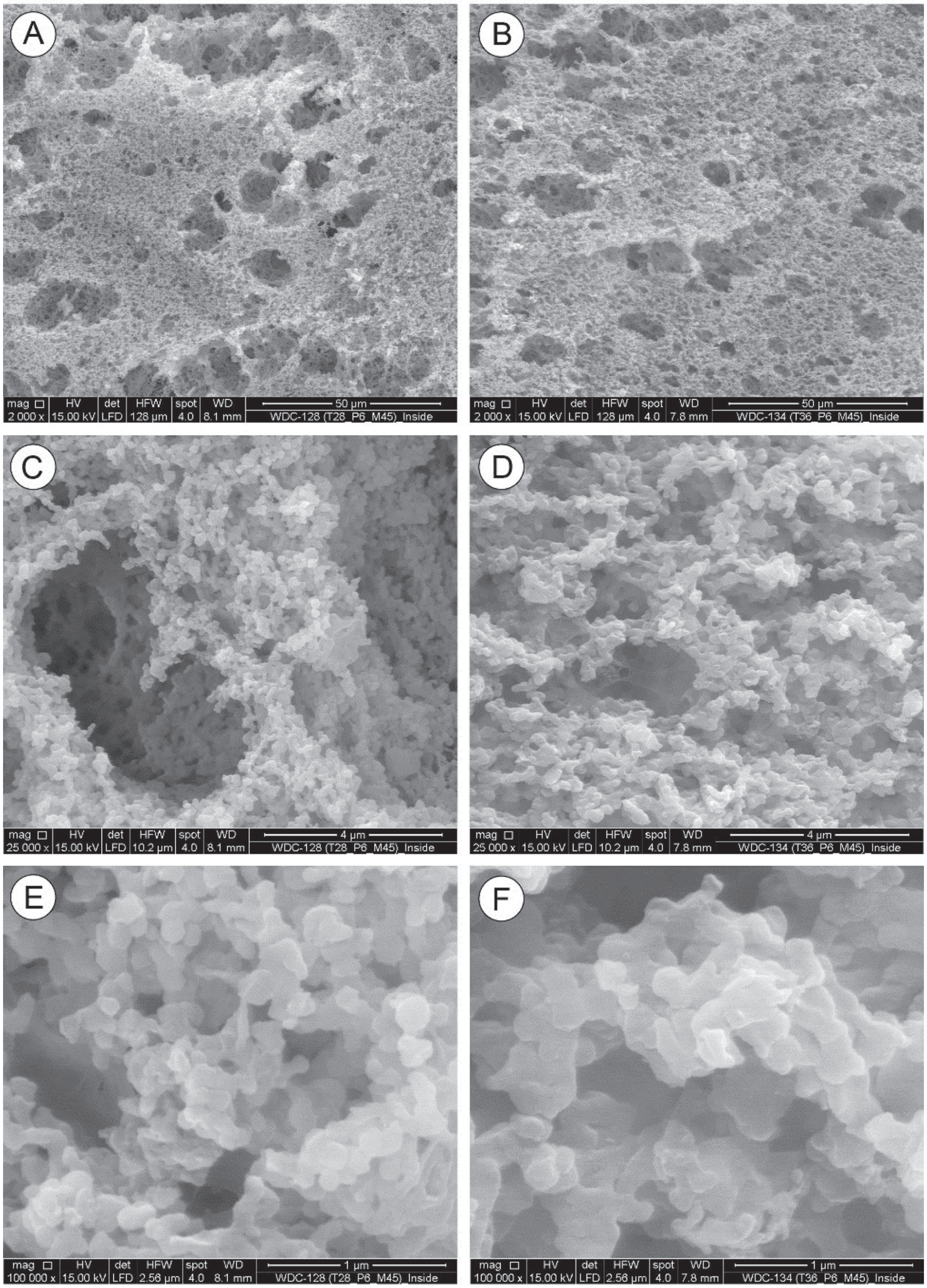

Figure 6. Representative scanning electron micrographs of cooked curd particles made from 6% protein-standardized milk that were renneted at 2 set temperatures (A, C, E: 28°C; B, D, F: 36°C). Micrographs were taken at a distance of approximately 500 µm from the outside of the particle.

loss property were presented. The trend for moisture loss from curd cubes of 6 mm was logarithmic, whereas moisture loss for larger curds was of a linear pattern. Curds set at lower temperature released less moisture immediately after cutting, which can be accelerated by reducing the cut size. Response surface modeling showed that cut size and protein levels were the most important factors influencing moisture content during stirring, whereas coagulation temperature did not have a significant influence, particularly after cooking (heating to 37°C). When protein concentration in the milk was increased, excessive curd breakage occurred when a large cut size (12–18 mm) was used. This suggested that cutting to smaller cut sizes would be required for cutting the coagulum from protein-concentrated milk. It was shown that overlaying permeate on the coagula from protein-concentrated milk will prevent breakage of some curds. However, further research is required to investigate the effect of the application of permeate on coagula on cheese quality and composition in an industrial cheese manufacturing process. This study shows that if the cut size cannot be increased during cheesemaking with milk of increased protein level, it is necessary to shorten the vat residence time before draining the whey. The microstructure of the cheese curd after cooking (as shown using both transmission and scanning electron microscopy) retained the differences initiated by coagulating the milk at different temperatures, with curd set at 36°C having thicker protein network strands with more fusion of casein

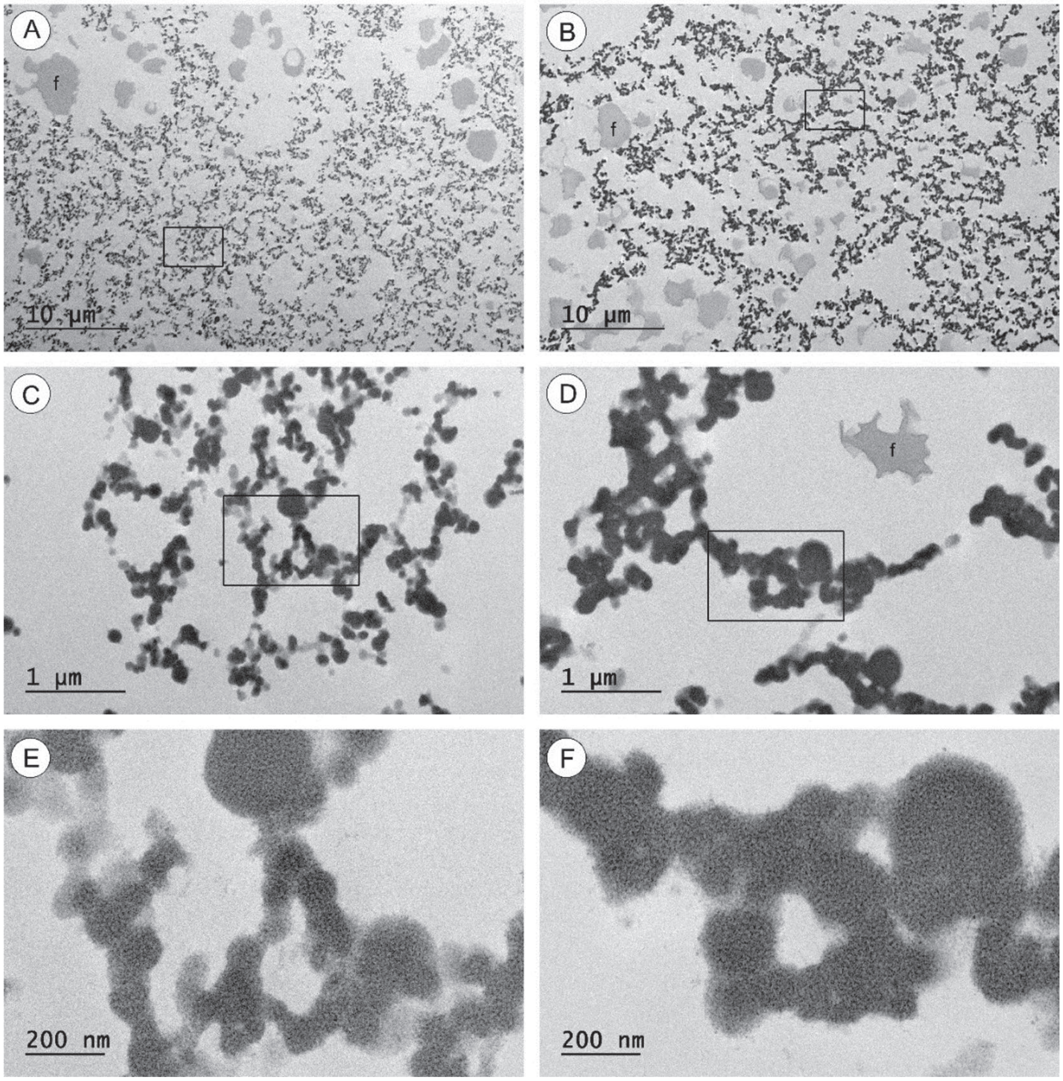

Figure 7. Internal microstructure of cooked curd particles manufactured with 6% protein-standardized milk renneted at different set temperatures (A, C, E: 28°C; B, D, F: 36°C) as analyzed by transmission electron microscopy. Micrographs were taken at a distance of approximately 500 to 600 µm from the outside of the particle. Boxed areas are shown in increasing magnifications. f = fat globules.

particles compared with curd coagulated at 28°C. A fundamental understanding of curd moisture loss properties as influenced by cut size, protein concentration, and coagulation temperature gained from this research can be applied in the cheesemaking process to predict curd moisture content from protein-standardized milk, reduce the effect of seasonal variability in milk composition, and increase the process efficiency.

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