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Effect of Cutting Time, Temperature, and Calcium on Curd Moisture, Whey Fat Losses, and Curd Yield by Response Surface Methodology

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ABSTRACT

Response surface methodology was used to study the effect of temperature, cutting time, and calcium chloride addition level on curd moisture content, whey fat losses, and curd yield. Coagulation and syneresis were continuously monitored using 2 optical sensors detecting light backscatter. The effect of the factors on the sensors' response was also examined. Retention of fat during cheese making was found to be a function of cutting time and temperature, whereas curd yield was found to be a function of those 2 factors and the level of calcium chloride addition. The main effect of temperature on curd moisture was to increase the rate at which whey was expelled. Temperature and calcium chloride addition level were also found to affect the light backscatter profile during coagulation whereas the light backscatter profile during syneresis was a function of temperature and cutting time. The results of this study suggest that there is an optimum firmness at which the gel should be cut to achieve maximum retention of fat and an optimum curd moisture content to maximize product yield and quality. It was determined that to maximize curd yield and quality, it is necessary to maximize firmness while avoiding rapid coarsening of the gel network and microsyneresis. These results could contribute to the optimization of the cheese-making process.
Key words: light backscatter, curd moisture, whey fat, curd yield

INTRODUCTION

Optimization of cheese moisture, yield, and quality has become increasingly important to the dairy industry. These parameters are strongly affected by the extent of syneresis and syneresis kinetics. Syneresis is caused by the contraction of the curd matrix due to rearrangement of the paracasein network bonds. The rate of syneresis is determined by the pressure gradient developed in the network and by the flow resistance through the gel network. The tendency of a milk gel to exhibit syneresis is mainly determined by milk composition and pretreatment, the coagulation conditions, and the rheological properties of the gel at cutting. Dejmek and Walstra (2004) noted that, because of the importance of the syneresis process in the manufacture of cheese, it is useful to understand and quantitatively describe syneresis as a function of milk properties and process conditions. A number of studies have been published in which the effect of various factors on the rate and extent of syneresis was investigated. These studies have investigated the effect of factors such as temperature (Marshall, 1982), curd firmness (Johnson, et al., 2001), calcium chloride addition (Marshall, 1982; McMahon et al., 1984; Lucey and Fox, 1993), pH (McMahon et al., 1984), and cutting procedure (Johnston et al., 1998). Table 1 summarizes the results of a number of studies on the effect of the factors investigated in this study (temperature, cutting time, calcium) and their proposed consequences on syneresis.

Syneresis is very temperature dependent. Temperature affects syneresis in 2 ways because both coagulation temperature and syneresis temperature affect the rate and extent of syneresis. Coagulation temperature changes the half-life and rate of formation of bonds (Lagoueyte et al., 1994; Lucey, 2002; Mishra et al., 2005), which strongly affects the rheological and microstructural properties of the gel at cutting. van Vliet et al. (1991) reported that, in rennet gels, an increase in the coagulation temperature in the range of 20 to 35°C raised gel permeability ($\boldsymbol{B}$) as well as dB/dt, endogenous syneresis pressure, and the one-dimensional shrinkage of gels. Syneresis temperature also affects the rate of whey separation (Lawrence, 1959; Marshall, 1982). van den Bijgaart (1988) found that dB/dt increased with increasing temperature. He stated that the faster increase of B with time at higher temperatures must be caused by easier breaking of bonds and

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Table 1. The effect of temperature (T), cutting time (t_{cut}) and calcium chloride addition level ($CCAL$) on curd syneresis

Parameter	Proposed mechanism	Microscopic consequence	Macroscopic consequence	Reference
Temperature				
Increasing; T < ~32°C	Increased strength or number of bonds	Increased fusion of casein micelles	Increased whey expelled Decreased TS released	Lagoueyte et al. (1994) Mishra et al. (2005)
Increasing; T > ~32°C	Decreased bond half life	Network susceptible to spontaneous breaking, rearrangement and coarsening	Increased whey released Increased TS released	Lagoueyte et al. (1994) Lucey (2002) Mishra et al. (2005)
Increasing; T > ~37°C	Melting of fat globules at >37°C	Increased mobility of fat globules	Increased fat released	Fox and McSweeney (2006) Fagan et al. (2007)
t_{cut}				
Late cut; t_{cut} > optimum	Increased strength or number of bonds Decreased rearrangement	Increased network rigidity	Decreased whey expelled Increased curd moisture	Mishra et al. (2005)
Early cut; t_{cut} < optimum	Decreased firmness	Disruption of fragile gel	Increased fat and TS released	Mishra et al. (2005)
$CCAL$				
Increasing; $CCAL$ < ~10 mM	Increased colloidal calcium phosphate	Increased linkages and firmness	Increased whey expelled	McMahon et al. (1984) Lucey and Fox (1993)
Increasing; $CCAL$ > ~10 mM	Increased ionic strength	Decreased firmness, weak gel	Decreased whey expelled	McMahon et al. (1984) Lucey and Fox (1993)

the disruption of junctions at greater temperatures. Therefore, the curd matrix has a greater capacity to rearrange and contract resulting in greater whey separation. The effect of calcium chloride addition on syneresis is more complex because addition of calcium chloride results in significant changes in ionic strength, colloidal calcium phosphate, and milk pH, which in turn affect both syneresis and the formation and strength of the casein gel. Small additions of calcium chloride (up to 10 mM) have generally been found to increase syneresis (Marshall, 1982), although some studies noted that small additions of calcium chloride had little or no effect on syneresis (Lawrence, 1959). At higher addition levels, the effect of calcium chloride has been observed to reduce syneresis.

A number of rheological and microstructural gel properties that are critical for adequate curd shrinkage and whey release (e.g., network rearrangement capability, coagulum firmness, permeability, and porosity) depend on appropriate cutting-time selection. As a result, cutting time greatly affects moisture, yield, and quality of cheese and whey fat losses. Johnston et al. (1998) also showed that cutting and stirring speeds could affect curd particle size and fat losses during syneresis. At constant cutting and stirring speeds, cutting the gel when it is too soft results in shattering of the fragile network, which increases curd fines and whey fat losses, thus, decreasing cheese yield (Lawrence, 1991). Although delaying the cutting time tends to induce the opposite effect, an excessive delay in cutting produces an overly firm gel, in which the network is unable to rearrange, which increases curd moisture content (Castillo et al., 2006b). Excessive moisture content, without the appropriate level of total solids retention, would result in an undesirable increase in yield, which could alter the ripening process, compromising cheese quality. Thus, there is likely an optimum time and hence firmness, for cutting the milk gel to optimize cheese yield and quality.

Although it is important to understand the effect of milk properties and processing conditions on curd shrinkage and whey release during cheese manufacturing, there is a lack of information on the effect of such factors and their interactions on syneresis. The first objective of this study was to investigate the effects of temperature, cutting time, and calcium chloride addition on cheese-making indices and on syneresis kinetics using response surface methodology. Light backscatter sensor technologies with a large field of view have proven successful for monitoring milk coagulation (Castillo et al., 2005) and syneresis (Fagan et al., 2007). Thus, a second objective was to monitor those 2 processing steps using a large field of view light backscatter sensor to study the effects of the experimental factors on the optical response and its derived parameters.

MATERIALS AND METHODS

Experimental Design

A 3-factor, fully randomized, spherical, central composite design (**CCD**) was used to study the effect of

Table 2. Experimental factors and their levels used in the central composite rotatable experimental design

Factors (coded value)	Temperature (°C)	Added $CaCl_2$ (m*M*)	Cutting time (β value, dimensionless)[1]
–1.682	23.6	0.32	1.32
–1	27.0	1.00	1.80
0	32.0	2.00	2.50
1	37.0	3.00	3.20
1.682	40.4	3.68	3.68

[1]Experimental cutting-time levels were selected as $\beta \times t^*_{max}$, where t^*_{max} was the time from enzyme addition to the inflection point of the light backscatter profile obtained using the CoAguLite sensor.

several significant coagulation and syneresis factors and gel properties at cutting on syneresis indices (e.g., curd yield, whey fat concentration, curd moisture content). The CCD consisted of a 2^k factorial ($k = 3$) with $2k$ axial points and 6 center points (i.e., 20 runs in total) and was carried out in triplicate. Coagulation temperature (***T***), calcium chloride addition level (***CCAL***), and cutting-time level (β) were selected as independent variables. The selected levels and coded values of the experimental factors are presented in Table 2. This experimental design allows for the estimation of curvature (second-order polynomial model) and can provide levels at which the independent variables will optimize a dependent variable.

Inline, continuous monitoring of milk coagulation and curd syneresis in a 7-L cheese vat was performed using 2 different light backscatter sensor technologies, the CoAguLite (**CL**) sensor (Reflectronics Inc., Lexington, KY) and the large field of view (**LFV**) sensor (Univ. Kentucky, Lexington). Experimental cutting-time levels were selected by light backscatter measurements using the CL sensor as described below.

Milk Preparation and Coagulation

Unpasteurized and unhomogenized milk obtained from a local Kentucky milk-processing plant was pasteurized at 65°C for 30 min. Milk was analyzed for fat, protein, and TS content using a MilkoScan FT 120 (Foss Electric, Hillerød, Denmark). The average composition of the milk was determined as 3.7 ± 0.3%, 3.5 ± 0.1%, and 12.2 ± 0.3% for fat, protein, and TS contents, respectively. Calcium chloride was added to 7.20 kg of milk at the required level, and the milk was stirred for 3 min. Milk was adjusted to a pH of 6.5 using the method described by Fagan et al. (2007). Milk pH adjustment after $CaCl_2$ addition ensured that any observed effect of calcium level on dependent variables was not due to an indirect effect of $CaCl_2$ on milk pH.

On the day of coagulation, milk was slowly heated to the target coagulation temperature ±0.15°C using a water bath. Seven kilograms of the heated milk was added to the vat and left to equilibrate until thermal equilibrium was achieved. Coagulation temperature was controlled by water circulation via a water bath with a control accuracy of ±0.01°C (Lauda, RM 20, Brinkmann Instrument Inc., Westbury, NY). Milk temperature was measured with a precision thermistor (model 5831A, Omega Engineering, Stanford, CT; resolution: ±0.01°C; accuracy: ±0.2°C). Chymosin (Chy-Max Extra; Chr. Hansen Inc., Milwaukee, WI) was added to 7 kg of milk in the vat at a level of 0.06 mL/kg of milk, and the milk was stirred for 1 min. Data acquisition for the CL and LFV sensors commenced upon addition of the enzyme; that is, at time t_{c0} (subscript "c" denotes the coagulation monitoring period).

Online Light Backscatter Monitoring Instrumentation

The CL sensor (model 5, Reflectronics Inc.) was used to select the different experimental levels of cutting time. This sensor used near-infrared radiation at 880 nm and consisted of two 600-μm-diameter fibers. One fiber transmitted infrared radiation into the milk sample and the other fiber transmitted the radiation scattered by the milk particles to a silicon photodetector. Further details on the CL sensor and data acquisition system were presented previously by Castillo et al. (2006a) and Fagan et al. (2007).

The LFV sensor was a prototype designed at the University of Kentucky to monitor coagulation and syneresis. This sensor uses near-infrared light at 980 nm, which is transmitted to the milk via a quartz rod, while a large-diameter glass window allows backscattered light to be collected over a large area and transmitted through a second quartz rod and a fiber optic cable to a miniature fiber optic spectrometer (model SD2000, Ocean Optics Inc., Dunedin, FL). Further details on the LFV sensor and data acquisition system were presented by Fagan et al. (2007).

The responses of the CL and LFV sensors were treated as follows. The initial voltage response ($\boldsymbol{V_0}$) was calculated by averaging the first minute of data. A light backscatter ratio (***R***) was calculated by dividing the sensor output voltage by V_0. The first derivative (R') of the light backscatter ratio profile was calculated by conducting linear least-squares regression on the most recently collected 4 min of data. The calculated slope was assigned to the midpoint of the data subset used. The second derivative (R'') was calculated in a similar manner but using 60 data points to smooth the R'' profile. A number of optical parameters were derived from both the LFV and CL sensor response

during coagulation and syneresis as outlined in Castillo et al. (2006a). The optical parameters investigated in this study are $\boldsymbol{t_{max}}$, the time to the first maximum of R'; $\boldsymbol{t_{2max}}$, the time to the first maximum of R''; $\boldsymbol{t_{2min}}$, the time to the first minimum of R''; and $\boldsymbol{\Delta R_{syn}}$, the percentage decrease in R from cutting time to the end of syneresis ($\boldsymbol{t_{s85}}$; subscript "s" denotes the syneresis monitoring period).

Selection of Cutting-Time Levels and Cutting Procedure

The CL sensor assisted the selection of the cutting time using the following equation proposed by Payne et al. (1993):

$$t_{cut} = \beta t^*_{max}, \quad [1]$$

where β was a constant and the asterisk indicates that t_{max} values used for cutting-time selection were derived from the CL sensor response. A number of different β values (1.3, 1.8, 2.5, 3.2, and 3.7), obtained in compliance with the experimental design shown in Table 2, were used to establish the range of target t_{cut} values for the experiment. Because an increase in β represents a delay in cutting, we will use the term "cutting time" ($\boldsymbol{t_{cut}}$) instead of the less intuitive symbol β during the discussion. The gel was cut when indicated by the CL data acquisition software. The curd was left to heal for 4.5 min before stirring (10 ± 0.02 rpm) was initiated (Servodyne mixer 50003-10, Cole Parmer Instrument Co., Vernon Hills, IL). The stirring process continued at this speed up to 85 min (t_{s85}).

Curd and Whey Sampling Procedure

Homogeneous samples of curd and whey (~150 mL) were removed for compositional analysis at 5 min from cutting ($\boldsymbol{t_{s5}}$) and every 10 min thereafter up to t_{s85} (i.e., 9 samples) according to the method described by Fagan et al. (2007) and Everard et al. (2007). Curd and whey samples were separated using a no. 200 stainless steel standard test sieve (Fisher Scientific, Hampton, NH) with a 75-μm absolute pore size. The sieve characteristics were selected to ensure that whey fat globules were not retained by the sieve.

Compositional Analysis of Curd and Whey and Calculation of Component Recoveries and Curd Yields

Approximately 3 g of curd and 5 g of whey were weighed into preweighed aluminum dishes using an analytical balance with a resolution of 0.1 mg ± 0.2 mg (AE260, Mettler-Toledo Inc., Columbus, OH) for determination of TS of curd and whey at each sampling time. The dishes were dried in a convection oven at 102°C until they reached a constant weight (~15 h). Each sample was analyzed in triplicate. Chemical composition of whey (fat, protein, and TS content) was determined in triplicate using a previously calibrated MilkoScan FT120 (Fagan et al., 2007). Curd moisture and whey fat contents (%) during syneresis were defined as $\boldsymbol{CM_t}$ and $\boldsymbol{WF_t}$, respectively, where t was the time from cutting at which the sample was taken. Total whey fat losses ($\boldsymbol{WFL}$, g) at the end of each experiment were calculated as shown in Table 3. The curd yield ($\boldsymbol{CY}$) for each experiment was computed using 2 equations as detailed in Table 3. Curd yield on a wet basis ($\boldsymbol{CY_{wb}}$) was influenced not only by the TS retained in the curd but also by the curd moisture content. Curd yield on a dry basis ($\boldsymbol{CY_{db}}$), however, provided information relating to the TS retained in the curd. The percentage of curd fat retention ($\boldsymbol{CFR}$) was also calculated as shown in Table 3.

Table 3. List of cheese-making indices evaluated in this study and equations for their calculation

Parameter	Calculation[1,2]
Total whey fat loses, g	$WFL = \frac{(M - C)\cdot F_w}{100}$
Curd yield, wet basis, %	$CY_{wb} = \frac{C}{M}100$
Curd yield, dry basis, %	$CY_{db} = \frac{C\cdot TS_c}{M\cdot TS_m}100$
Curd fat retention, %	$CFR = \frac{M\cdot F_m - W\cdot F_w}{M\cdot F_m}100$

[1] C = curd weight (g); M = milk weight (g); W = whey weight (g); F_m = milk fat (%); F_w = whey fat (%); TS_c = curd TS (%); TS_m = milk TS (%).

[2] Values used for parameters F_w, and TS_c corresponded to recorded values at t = 85 min.

Statistical Analysis

Statistical analysis was carried out using the RSREG procedure of SAS (version 9.1, 2002–2003; SAS Institute, Cary, NC) to determine significant differences ($P < 0.05$). The RSREG procedure uses the method of least squares to fit quadratic response surface regression models and to obtain information about the fit in the form of an ANOVA. Response surface regression models were generated for each of the dependent variables. Three-dimensional response surface plots of each predicted parameter were also generated.

Table 4. Analysis of variance[1] and *F*-statistic for the syneresis indices

	Syneresis indices[2]							
Factors	WF_5	WF_{85}	*WFL*	CM_5	CM_{85}	CY_{wb}	CY_{db}	*CFR*
T	–0.38***	–0.47***	–19***	2.5**	-1.1^{NS}	–1.3*	2.0*	11***
Cutting time (β)	–0.82**	–0.39*	–17*	1.7^{NS}	0.35^{NS}	2.8^{NS}	0.29^{NS}	1.4^{NS}
CCAL	–0.61**	-0.03^{NS}	-1.6^{NS}	1.1^{NS}	0.22^{NS}	2.6^{NS}	0.51^{NS}	0.51^{NS}
$\beta \times \beta$	0.03^{NS}	-0.005^{NS}	-0.22^{NS}	0.16^{NS}	-0.32^{NS}	0.05^{NS}	0.29^{NS}	0.80^{NS}
$\beta \times T$	0.02*	0.01**	0.55**	-0.12^{NS}	0.05^{NS}	-0.05^{NS}	-0.49^{NS}	–2.4**
$T \times T$	0.004***	0.007***	0.29***	–0.04**	-0.008^{NS}	0.01^{NS}	-1.9^{NS}	–12***
$CCAL \times \beta$	0.03^{NS}	0.01^{NS}	0.92^{NS}	0.55^{NS}	-0.22^{NS}	–0.70*	-0.91^{NS}	-1.2^{NS}
$CCAL \times T$	0.01**	-0.0002^{NS}	-0.03^{NS}	-0.08^{NS}	0.05^{NS}	0.02^{NS}	0.25^{NS}	-0.38^{NS}
$CCAL \times CCAL$	0.02^{NS}	-0.003^{NS}	-0.04^{NS}	-0.02^{NS}	-0.42^{NS}	-0.28^{NS}	-0.23^{NS}	0.72^{NS}
R^2	0.84	0.92	0.93	0.72	0.95	0.91	0.21	0.88
F	22***	50***	56***	11***	82***	40***	1.4^{NS}	38***

[1]df = 11; n = 60; R^2 = determination coefficient; *F* = ANOVA *F*-statistic; *T* = temperature; β = a constant as defined by the experimental design and used in Eq. [1] to establish the experimental cutting time; *CCAL* = calcium chloride addition level; × denotes interaction of experimental factors.

[2]WF_5 = whey fat content at 5 min after cutting; WF_{85} = whey fat content at 85 min after cutting; *WFL* = total whey fat losses (g) at the end of each experiment; CM_5 = curd moisture at 5 min after cutting; CM_{85} = curd moisture at 85 min after cutting; CY_{wb} = curd yield on a wet basis; CY_{db} = curd yield on a dry basis; *CFR* = curd fat retention;

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; NS = not significant.

RESULTS

Effect of Temperature, $CaCl_2$, and Cutting Time on Component Losses and Curd Yield

The effect of independent variables on component losses and recoveries and curd yields was investigated using the RSREG procedure in SAS. The results of the ANOVA are shown in Table 4 for WF_5, WF_{85}, *WFL*, CM_5, CM_{85}, CY_{wb}, CY_{db}, and *CFR* models. All of the models except CY_{db} were highly significant in their fit ($R^2 \geq 0.72$, $P < 0.001$). Models WF_{85} and *WFL* were significantly affected by temperature and temperature-squared ($P < 0.0001$) as well as by the cutting time ($P = 0.03$) and temperature-cutting time interaction terms ($P = 0.004$). The results of the ANOVA for WF_{15}, WF_{25}, WF_{35}, WF_{45}, WF_{55}, WF_{65}, and WF_{75} (not shown) were the same as for WF_{85}. For WF_5, *CCAL* and the *CCAL*-temperature interaction terms were also significant ($P < 0.006$). This suggests that *CCAL* will predominantly affect the concentration of fat in the whey in the early stages of syneresis. van den Bijgaart (1988) stated that adding calcium up to 4.5 m*M* enhances the rate of aggregation but also leads to a faster increase in the rigidity of the network. Initially, increased aggregation leads to higher initial syneresis pressure, but the increase in pressure is hindered at an earlier stage due to reduced rearrangement. Therefore, the addition of calcium tended to increase the initial curd shrinkage rate. In the case of the CM_{85} ANOVA model, no term except the intercept ($P < 0.0001$) was found to be significant. However, the temperature and temperature-squared term did significantly ($P < 0.02$) affect CM_5 (Table 4) and CM_{15}, CM_{25}, CM_{35}, CM_{45}, CM_{55}, and CM_{65} (results not shown).

Both CY_{wb} and CY_{db} were significantly affected by temperature ($P < 0.01$). The calcium chloride-cutting time interaction term was found to be significant for CY_{wb} ($P < 0.05$). Temperature and cutting time were significant in determining the retention of fat in the curd with temperature, temperature-squared, and the temperature-cutting time interaction terms all found to be significant for *CFR* ($P < 0.01$).

The response surface graphs for *WFL*, CM_{85}, and CY_{wb} as a function of the independent variables are shown in Figure 1. The *WFL* was minimized between 28 and 30°C depending on t_{cut} (Figures 1a and b). Figure 1b graphically shows the significant interaction existing between t_{cut} and temperature ($P < 0.01$; Table 4). It shows that, at lower temperatures, decreasing t_{cut} increased *WFL*, whereas at high temperatures, decreasing t_{cut} decreased *WFL*. At an early t_{cut} ($\beta = 1.8$), the optimum temperature for minimizing *WFL* was 30°C, but at later t_{cut} ($\beta = 3.7$), *WFL* was minimized at 28°C. Figure 1c confirms that increasing β (i.e., t_{cut}) at 33°C increased *WFL*. This finding may have practical implications for cheese processing. It suggests that delaying cutting time at this typical cheese-making temperature would tend to increase fat losses. Figure 1c also indicates that there may be an interactive effect between *CCAL* and t_{cut} on *WFL* at 33°C. At early t_{cut}, increasing *CCAL* decreased fat losses whereas the opposite effect was observed at late t_{cut}.

Figure 1d and 1e show the effect of temperature on CM_{85} although this effect was not found to be signifi-

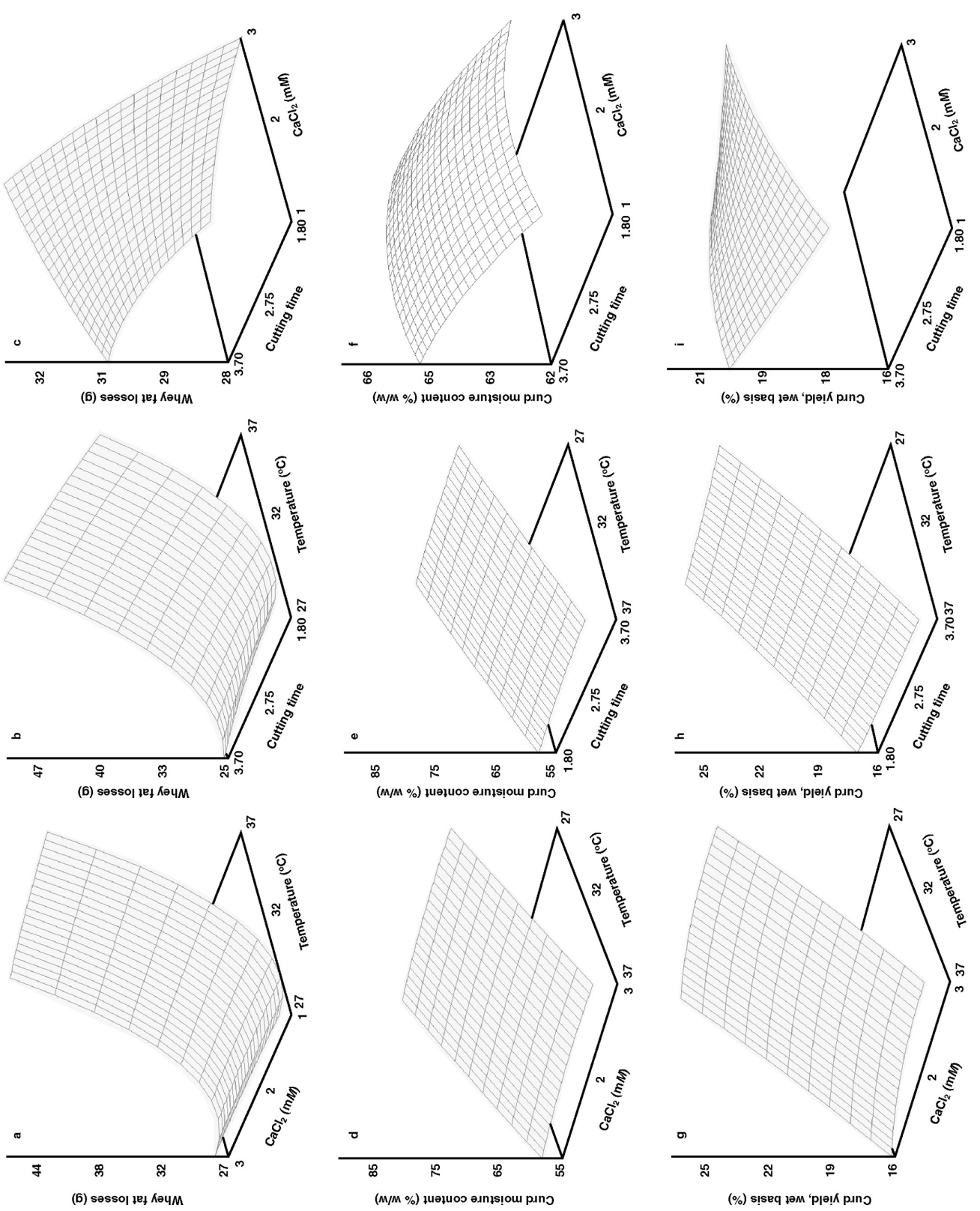

Figure 1. Response surface plots for the effect of independent variables $CaCl_2$ addition level, temperature, and cutting time on total whey fat losses (a, b, c), curd moisture content at 85 min after cutting (d, e, f), and curd yield on a wet basis (g, h, i).

cant ($P = 0.07$). Decreasing temperature was shown to increase CM_{85}. In Figure 1e and 1f, increasing t_{cut} was found to increase CM_{85}. The *CCAL* was observed to have a limited effect on CM_{85} at 33°C (Figure 1f) with CM_{85} maximized at ~1.8 m*M*.

The effect of temperature on CY_{wb} is shown in Figures 1g and 1h. Decreasing the temperature was shown to increase CY_{wb}; this is related to the reduced extent of syneresis. The significant interactive effect ($P < 0.05$; Table 4) of *CCAL* and t_{cut} on CY_{wb} is shown in panel i of Figure 1. At low *CCAL*, increasing t_{cut} increased CY_{wb}, whereas at high *CCAL*, increasing t_{cut} reduced CY_{wb}. The effect of t_{cut} also differed depending on the *CCAL*. At earlier t_{cut}, increasing the *CCAL* increased CY_{wb}; the opposite effect was observed in later-cut gels.

Figure 2 shows the response surface graphs for CY_{db} and *CFR* as a function of the independent variables. The effect of temperature on CY_{db} can be seen in Figures 2a and b. The CY_{db} was maximized at 33°C for low *CCAL* (1 to 1.2 m*M*) and at 34°C for higher *CCAL* (1.3 to 3 m*M*) in Figure 2a and maximized at 35°C (Figure 2b) for early t_{cut} ($\beta = 1.8$ to 2) and 33°C at later t_{cut} ($\beta = 3$ to 3.7). Therefore, as was observed for *WFL* (Figure 1b), varying t_{cut} shifted the temperature at which the maximum level of TS was retained, with the maximum CY_{db} occurring at a higher temperature (35°C) when t_{cut} was earlier (Figure 2b).

The results obtained for *CFR* also support the results observed for WF_{85} (Figure 1a, b, and c). The *CFR* was maximized in the temperature range 29 to 31°C (Figures 2d and e), with the levels of *CFR* decreasing rapidly with increasing temperature (Figure 2d and e). Terms *CCAL* and t_{cut} also affected the fat retention capacity of curd particles. The effect of high *CCAL* and later t_{cut} resulted in curd particles that had a reduced ability to retain fat (Figure 2f).

Effect of Temperature, $CaCl_2$, and Cutting Time on the LFV Light Backscatter Parameters

The effect of independent variables on light backscatter parameters derived from the LFV signal was also investigated using the RSREG procedure in SAS. The results of the ANOVA are shown in Table 5. The fit for the t_{max}, t_{2max}, and t_{2min} models were higher ($R^2 \geq 0.88$, $P < 0.001$), than for the ΔR_{syn} model ($R^2 = 0.68$, $P < 0.001$). The effect of t_{cut} on t_{max}, t_{2max}, and t_{2min} was not included in Table 5 because these parameters were determined during milk coagulation, before cutting the gel. As expected, t_{max}, t_{2max}, and t_{2min} were strongly affected by temperature and the temperature-squared term ($P < 0.001$), due to the higher rate of enzymatic hydrolysis of κ-casein (O'Callaghan et al., 2001) and the casein micelle network assembly (Castillo et al., 2006a). Time parameters t_{2max} and t_{2min} were significantly affected by the temperature-*CCAL* interaction term ($P < 0.03$). The *CCAL* has previously been shown to have a direct effect on aggregation and firming rates (Castillo et al., 2002). The percentage decrease in *R* during syneresis (ΔR_{syn}) was significantly affected by temperature, the temperature-squared term, cutting time, and the cutting time-squared term.

The response surface graphs for t_{max}, t_{2max}, t_{2min}, and ΔR_{syn} as a function of the independent variables are shown in Figure 3. As expected, the response surface graphs for t_{max}, t_{2max}, and t_{2min} (Figure 3a, b, c) were very similar. In all cases, because of the effect of temperature on enzymatic hydrolysis and casein micelle aggregation, decreasing temperature increased each of the time parameters. This is in agreement with the results of Castillo et al. (2006a), who claimed that time parameters t_{max}, t_{2max}, and t_{2min} decreased with increasing temperature. It is also observed in Figure 3a, b, and c that the response of time parameters to temperature was not entirely linear, with the rate of change in these parameters decreasing with increasing temperature.

The effect of *CCAL* on all time parameters was consistent. Although *CCAL* had a limited effect at high temperature, increasing *CCAL* decreased t_{max}, t_{2min}, and t_{2max} at low temperatures due to faster aggregation and curd firming rates. This is in agreement with results presented by Castillo et al. (2002) and Najera et al. (2003). This may be due to the large effect that temperature has on aggregation, masking the effect of *CCAL* at high temperatures.

In the response surface graphs for ΔR_{syn} (Figure 3d, e, and f), temperature and t_{cut} maximized ΔR_{syn} from 31 to 32°C and from 2.6 to 1.8 (i.e., $\beta = 2.6$ to 1.8), respectively. It was also noted that at high *CCAL* (3 m*M*), the maximum ΔR_{syn} occurred at the shortest t_{cut} ($\beta = 1.8$).

DISCUSSION

Rheological and Microstructural Properties of Milk Gels at Cutting

It can be concluded from the current results that there is an optimum gel firmness at which the gel should be cut to achieve maximum retention of fat as well as an optimum curd moisture content that will maximize product yield and quality. The development of gel firmness and network rearrangement during the aging of casein gels before cutting, and the complex interactions existing among firmness, rearrangement capability, and processing factors such as gelation temperature, $CaCl_2$ addition, and cutting time can be

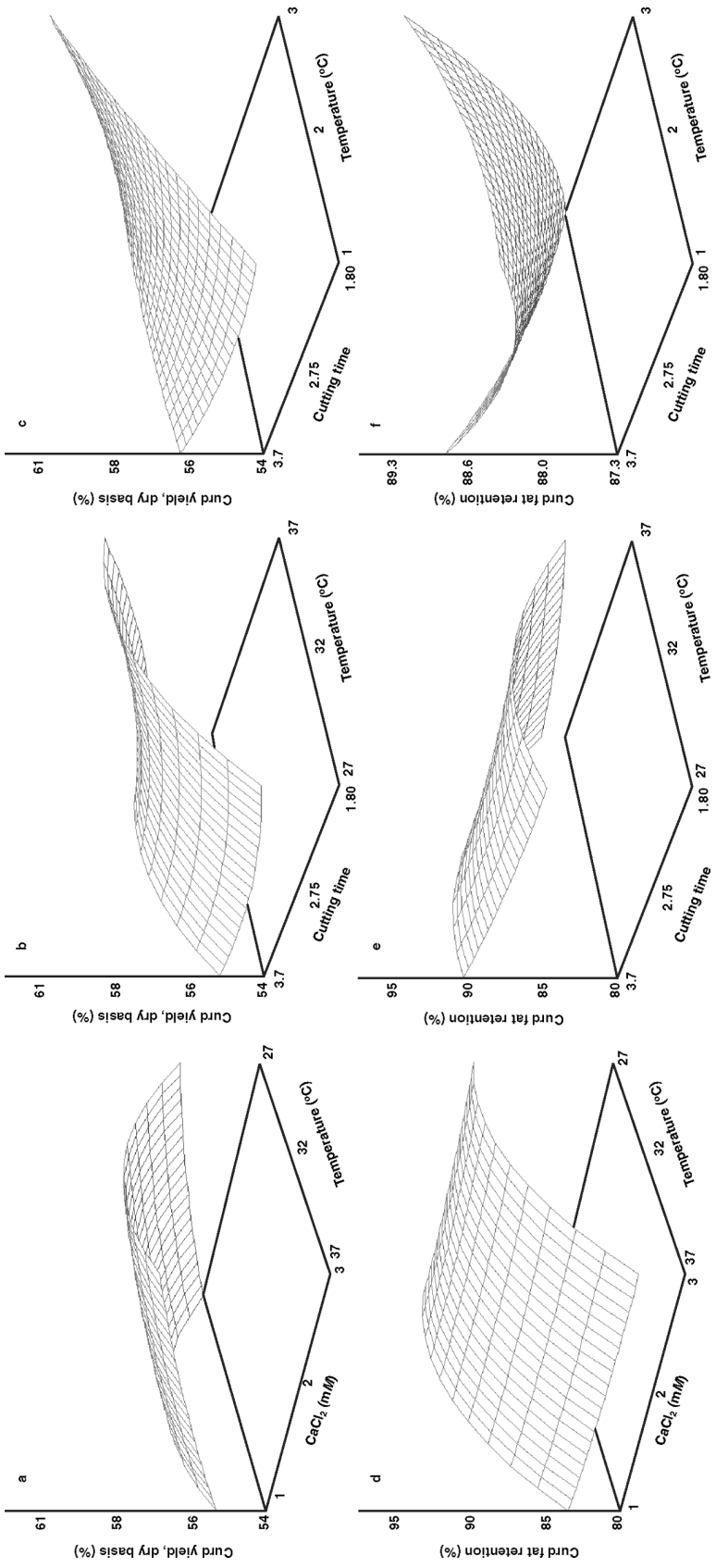

Figure 2. Response surface plots for the effect of independent variables $CaCl_2$ addition level, temperature, and cutting time on curd yield on a dry basis (a, b, c) and curd fat retention (d, e, f).

Table 5. Analysis of variance[1] and *F*-statistic for the light backscatter parameters

Factor	Light backscatter parameters[2]			
	t_{max}	t_{2max}	t_{2min}	ΔR_{syn}
T	–3.2***	–2.1***	–4.3***	20***
Cutting time (β)	NA	NA	NA	28*
CCAL	–0.87^{NS}	–2.1^{NS}	–1.6^{NS}	2.7^{NS}
$\beta \times \beta$	NA	NA	NA	–3.38*
$\beta \times T$	NA	NA	NA	–0.30^{NS}
$T \times T$	0.04***	0.02***	0.058***	–0.31***
$CCAL \times \beta$	NA	NA	NA	–1.25^{NS}
$CCAL \times T$	0.07^{NS}	0.09*	0.10*	0.08^{NS}
$CCAL \times CCAL$	–0.15^{NS}	–0.88^{NS}	–0.16^{NS}	–0.98^{NS}
R^2	0.90	0.88	0.89	0.68
F	41***	30***	33***	9.4***

[1]df = 11; n = 60; R^2 = determination coefficient; *F* = ANOVA *F*-statistic; *T* = temperature; β = a constant as defined by the experimental design and used in Eq. [1] to establish the experimental cutting time; *CCAL* = calcium chloride addition level; × denotes interaction of experimental factors.

[2]t_{max} = time to the first maximum of R'; t_{2max} = time to the first maximum of R'' ; t_{2min} = time to the first minimum of R''; ΔR_{syn} = percentage decrease in R from $t_{s(0)}$ to $t_{s(85)}$; NA = not applicable.

***$P < 0.001$; *$P < 0.05$; NS = not significant.

used to explain a number of mechanisms that lead to the results observed in Figures 1, 2, and 3. However, before addressing the analysis of the results of this study, a short review of the relationships among parameters such as temperature and aging time and rheological and microstructural properties of rennet induced milk gels is warranted.

It is known that the storage modulus (**G′**) of the gel will increase after gelation until (if left for a long enough period) it reaches a plateau; that is, ultimate firmness (Zoon et al., 1988; Dejmek and Walstra, 2004). Under certain circumstances, if the gel is not cut, G′ may then subsequently decrease (Zoon et al., 1988) due to microsyneresis, which occurs when regions of the gel become dense and whey is forced into pores that grow in diameter. Although the development of G′ with time is clear, there is some disagreement regarding the effect of temperature on curd firmness. Several authors have found that the rate of network formation is temperature dependent. Sharma et al. (1993) found that aggregation rate and curd firmness increased with increasing temperature. Although there is consensus among authors regarding the effect of temperature on both aggregation and curd firming rate constants, there is some disagreement regarding the effect of temperature on ultimate firmness. Some authors have stated that the final rigidity of rennet-induced milk gels increases with temperature (Bohlin et al., 1984), whereas others found that the maximum firmness of milk gels decreases with increasing temperature (Zoon et al., 1988). This is due to G′ being dependent not only on temperature, but also on time. If the G′ value of the milk gels is recorded after the gels have reached ultimate firmness (i.e., they have reached a plateau), then it is clear that the firmness of the gels will decrease with increasing temperature (Zoon et al., 1988). Therefore, while G′ initially increases more rapidly at higher temperatures, it will reach a maximum value more quickly and that value will be smaller than at lower temperatures (Zoon et al., 1988). This was also observed by Lagoueyte et al. (1994) who found that ultimate maximum firmness decreased with increasing temperature, but at aging times less than 4 h, the opposite was found to be true. This finding was further confirmed by Mellema et al. (2002), who stated that as long as the gel was in the early stages of aging, G′ is larger at higher temperatures. This occurred for the experimental conditions in this study, where the gel was cut within a time range of 9 to 48 min from enzyme addition.

Zoon et al. (1988) also found that at higher temperatures the rate of change in permeability with time is greater, the maximum endogenous syneresis pressure is reached more quickly after rennet addition, and the maximum endogenous syneresis pressure increased with increasing temperature. These factors were said to result from a rapid coarsening of the network. Coarsening results from the spontaneous breaking and rearranging of the network. As strands rearrange they form new strands and clusters that become denser and thicker, resulting in a more open network with larger pores and, hence, greater permeability (Lagoueyte et al., 1994). It has been widely observed that this change in permeability over time occurs more quickly in gels formed at higher temperatures, which will also have a greater final permeability (van den Bijgaart, 1988; Lagoueyte et al., 1994). This is due to the shorter half-life of the bonds formed at higher temperatures. The shorter half-life means that breaking of bonds and disruption of junctions occurs more easily. van den Bijgaart (1988) suggested that this indicated a more rapid decrease in the number of stress-containing junctions at higher temperatures, which could be interpreted as a relatively quick change to junctions with a high number of bonds. During aging, this rearrangement (which leads to denser aggregates and larger pores) can lead to tensile stress in nearby strands. Therefore, strands will be stretched and become thinner. Over a long aging time, this can cause strands to break, which forces whey out of specific regions of the gel that became denser, and into the growing pores. This is termed microsyneresis and it may result in a decrease in gel firmness.

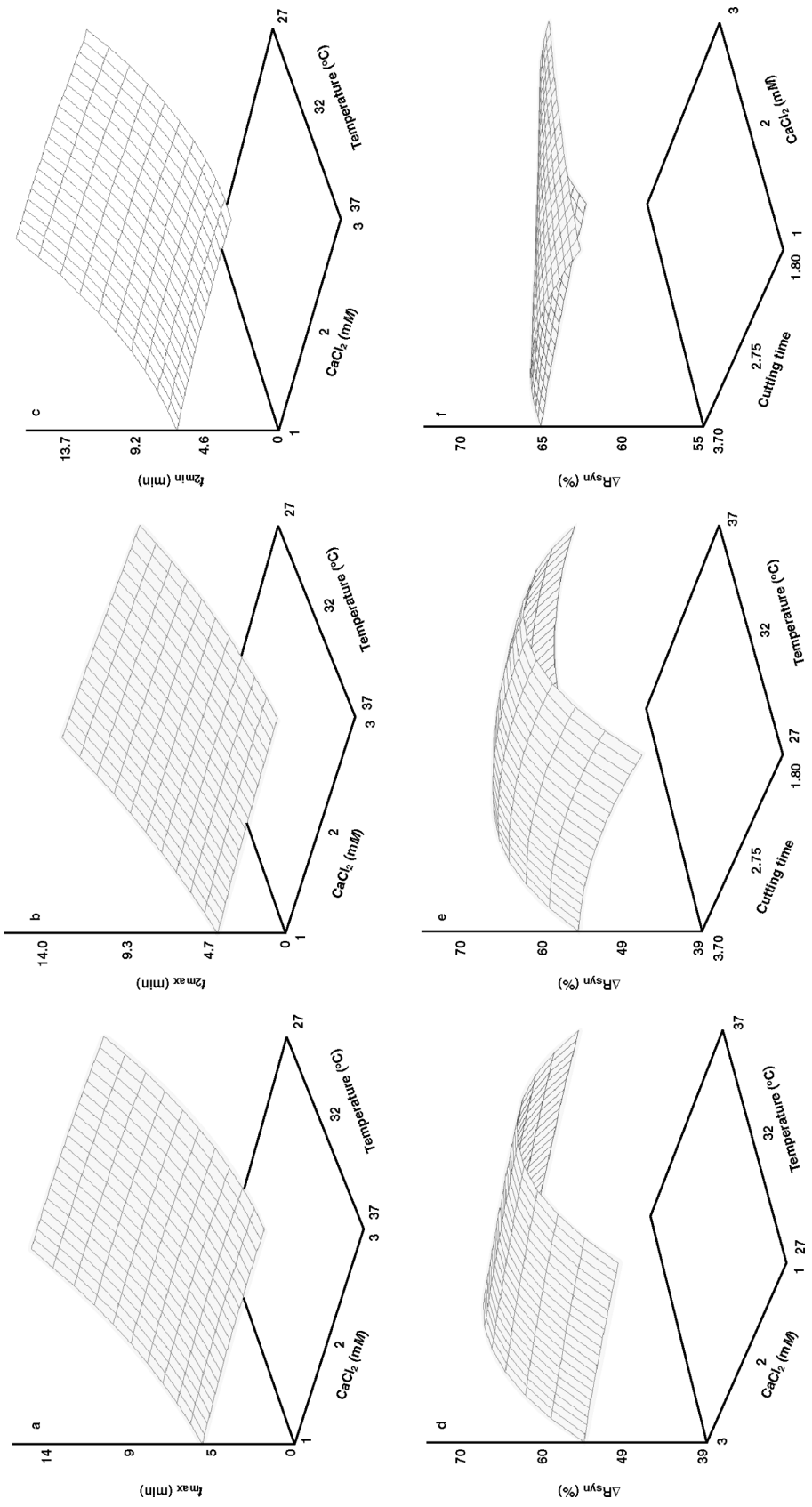

Figure 3. Response surface plots for the effect of independent variables $CaCl_2$ addition level, temperature, and cutting time on optical parameters derived from the large field of view light backscatter sensor: t_{max} (a), t_{2max} (b), t_{2min} (c) and ΔR_{syn} (d, e, f), where t_{max} = time to the first maximum of R'; t_{2max} = time to the first maximum of R''; t_{2min} = time to the first minimum of R''; and ΔR_{syn} = percentage decrease in R from $t_{s(0)}$ to $t_{s(85)}$.

Optimizing Curd Firmness at Cutting Affects Curd Moisture and Fat Losses

The result of not optimizing firmness on fat losses was seen in Figure 1a, b, and c for *WFL* and in Figure 2d, e, and f for *CFR*. As expected, the response surface graphs for *WFL* and *CFR* displayed a similar trend. The *WFL* and *CFR* were minimized and maximized, respectively, between 28 and 35°C, because this was the temperature range at which a gel with optimum firmness was formed over the range of t_{cut} and *CCAL* investigated. Our data shows, as suggested by Johnson et al. (2001), that the retention of fat is dependent on relative rigidity and structure of the network at cutting. At temperatures below 28°C, the gel will not have reached its ultimate firmness (i.e., plateau in G′) before cutting. Therefore, increasing the temperature increased the rate of curd firming and a firmer gel was formed as the temperature reached 35°C. At temperatures below 28°C, the gel was fragile when cut, which results in high levels of curd fines and fat losses. As the temperature increases, curd firmness at cutting reaches an optimum and fat losses are minimized. Above 35°C, the network becomes more rigid, rapid coarsening occurs, and the gel has a greater porosity, all of which assist the release of fat. Indeed, because the average melting point of milk fat is 37°C (Fox and McSweeney, 2006), fat has much greater mobility at higher temperatures approaching 37°C, which results in even greater levels of fat being released.

This effect of firmness also explains the interactive effect of temperature and t_{cut} on *WFL* and *CFR* (Figures 1b and 2e). At lower temperatures (<28°C) the gel will be relatively weak when cut. Therefore, increasing t_{cut} at temperatures up to 28°C results in a firmer gel with a better fat retention capacity. Consequently, increasing t_{cut} at low temperatures decreased *WFL* (Figure 1b). However, at higher temperature (>30°C), coarsening of the milk gel occurs more rapidly, permeability of the gel is greater, and microsyneresis can occur at longer aging times. All of these factors reduce the ability of the curd to retain fat. In this case, increasing t_{cut} will only exacerbate these processes; hence, at temperatures >30°C, increasing t_{cut} resulted in greater *WFL*.

The importance of combining the correct t_{cut} and *CCAL* to maximize fat retention is seen in Figures 1c and 2f. These figures represent the effect of t_{cut} and *CCAL* on fat losses at a constant temperature of 33°C. We observed that, at 33°C, the gel would have reached a suitable firmness. Consequently, the highest *WFL* and lowest *CFR* occurred at the longest t_{cut} (β = 3.7) and highest *CCAL* (3 m*M*; Figures 1c and 2f), all of which contributed to exceed the optimum firmness of the gel, which would result in microsyneresis, increased porosity, and greater fat losses.

Increasing temperature decreased CM_{85} (Figure 1d and e) because gels formed at higher temperatures have increased rearrangement capability (larger tan δ) and greater permeability, which results in a faster rate of syneresis and a larger amount of whey separation (Castillo et al., 2006b). The effect of increasing t_{cut} on increasing CM_{85} (Figure 1e and f) is in agreement with a number of previous studies (Johnson et al., 2001). This can be attributed to the firmer gels having a reduced capacity to rearrange and shrink, and thus a reduced ability to expel whey. The *CCAL* was observed to have a small effect on CM_{85} (Figure 1f) with CM_{85} maximized at ~1.8 m*M*. van den Bijgaart (1988) also found that *CCAL*, with correction for pH changes, had a limited effect on the syneresis rate. Calcium addition enhances aggregation and produces a higher initial syneresis pressure; however, the addition of calcium will also lead to a more rapid increase in the rigidity of the curd, and hence, a decrease in rearrangement and a decrease in syneresis (van den Bijgaart, 1988), which may explain the initial increase in CM_{85} with increasing *CCAL* (1 to 1.8 m*M*; Figure 1f). Although van den Bijgaart (1988) found that gel permeability was not affected by *CCAL*, he stated that this was somewhat surprising because the addition of calcium chloride increases the amount of calcium bound to the micelles, which, in turn, decreases their voluminosity. A decrease in the size of the gel building blocks may be expected to increase gel permeability, therefore increasing syneresis, which may account for the decrease in CM_{85} at *CCAL* >1.8 m*M* (Figure 1f).

Retaining Adequate Moisture and TS to Maximize Curd Yield and Quality

Lawrence et al. (1993) stated that cheese yield cannot be considered in isolation, but only in relation to the overall quality of the cheese. Although increasing the moisture content of cheese will increase the yield, unless it is associated with a greater retention of casein and fat, it may result in a decrease of quality through greater water activity, which will affect the ripening process, microbiological quality, and the development of flavor and texture. Therefore, to maximize cheese yield and quality, the retention of moisture and TS must be optimized.

Decreasing temperature increased CY_{wb} (Figure 1g and h) due to the reduced extent of syneresis resulting in higher curd moisture contents (Figure 1d and e). However, CY_{db} tended to be maximized between 33 and 35°C depending on *CCAL* and t_{cut} (Figures 2a and b) because gels below this temperature range would

tend to be weak and produce a fine curd when cut resulting in higher levels of fat and fines losses. However, at higher temperatures we noted much higher levels of fat losses (Figure 1a), which decreased CY_{db} (Figure 2a). The significant interactive effect of *CCAL* and t_{cut} on CY_{wb} ($P < 0.05$; Table 4 and Figure 1i) may be explained through the retention of casein and TS as well as moisture. This figure (Figure 1i) is representative of the effect of *CCAL* and t_{cut} on CY_{wb} at 33°C. Therefore, the weakest gel in this figure is formed at the lowest *CCAL* (1 m*M*) and t_{cut} ($\beta = 1.8$) levels, which results in higher losses of casein, fines, and fat (Figure 2c), as well as a low moisture content (Figure 1f), and hence the lowest CY_{wb}. This is confirmed in Figure 2c, in which a low CY_{db} (low retention of TS) is observed at the lowest *CCAL* and t_{cut}. In fact, it would seem that there is a range of *CCAL* and t_{cut} levels across which CY_{wb} is maximized (Figure 1i). This is observed as CY_{wb} maximized diagonally across Figure 1i from $t_{cut} = 3.7$ and *CCAL* = 1 m*M* to $t_{cut} = 1.8$ and *CCAL* = 3 m*M*. Increasing *CCAL* at early t_{cut} (1.8) increased curd yield (Figures 1i and 2c) through a combination of TS and moisture retention. This suggests that *CCAL* is compensating for the slightly less firm gel at low t_{cut} by increasing the firmness of the gel; hence, there is greater retention of TS; that is, CY_{db} (Figure 2c) including fat (Figure 1c). It should also be noted that CY_{wb} increases rapidly at short t_{cut} (1.8) when *CCAL* is increased from 1 to 2 m*M* but this increase slows as *CCAL* increases from 2 to 3 m*M* (Figure 2i). Although both TS (Figure 1c and 2c) and moisture (Figure 1f) are retained when *CCAL* increases from 1 to 2 m*M*, curd moisture will begin to decrease again if additional $CaCl_2$ is added (Figure 2f); hence, CY_{wb}, at short t_{cut} (1.8), starts to level off when *CCAL* increases to 3 m*M* (Figure 2i). Increasing t_{cut} at low *CCAL* levels has a compensatory effect on CY_{wb} mainly through retention of moisture. We have observed that, at 33°C, increasing *CCAL* increased CM_{85} (Figure 1f; from 1 to 2 m*M*) but also increased *WFL* (Figure 1c). At low *CCAL*, increasing t_{cut} had a minimal effect on overall CY_{db} (Figure 2c). Therefore, the observed increase in CY_{wb} when t_{cut} is lengthened at low *CCAL* (Figure 1i) can be attributed to an undesirable increase in curd yield; that is, increased moisture retention without an increase in TS retention. We noted that the highest *WFL* occurred at the longest t_{cut} (3.7) and highest *CCAL* (3 m*M*; Figure 1c). This was also observed in the lower CY_{db} obtained under the same conditions in Figure 2c. By employing these conditions (*CCAL* = 3 m*M* and $t_{cut} = 3.7$ at 33°C), the firmness and rigidity of the gel is maximized and rapid coarsening and microsyneresis can occur, which results in higher *WFL* reducing the CY_{db} (Figure 2c) and affecting CY_{wb} (Figure 1i).

These results indicate that within an optimal temperature range, possibly 28 to 35°C, a shorter t_{cut} and higher *CCAL* will maximize the retention of TS, casein, and fat, but reduce curd moisture content, whereas a later t_{cut} and lower *CCAL* will lower retention of TS but increase curd moisture. Therefore, to maximize both curd yield and quality it is necessary to select a combination of conditions that will maximize firmness while avoiding microsyneresis.

Relationship Between Light Backscatter Signal from the LFV and Syneresis

Increasing temperature decreased the coagulation time parameters, t_{max}, t_{2max}, and t_{2min} (Figure 3a, b, c). This is due to the effect that temperature has on both the primary (enzymatic hydrolysis) and secondary (aggregation reaction) phases of coagulation (Castillo et al., 2003). The decrease in the rate of change in these parameters with increasing temperature is in agreement with previously reported results. Castillo et al. (2003) stated that many authors, including Zoon et al. (1988), found a nonlinear effect of temperature on clotting time. This nonlinear effect may be related to the higher temperature coefficient ($\boldsymbol{Q_{10}}$) values for coagulation between 20 and 30°C than between 30 and 40°C. Castillo et al. (2003) suggested that this nonlinear effect might be due to heat inactivation of chymosin with increasing temperature. The Q_{10} value for coagulation may also explain the greater observed affect of *CCAL* on time parameters at low temperatures rather than at high temperatures (Figure 3a, b, c). It is known that although temperature will affect both phases of coagulation, the Q_{10} values for aggregation (11 to 30) are much higher than those reported for enzymatic hydrolysis (1.3 to 2), indicating that aggregation is more sensitive to changes in temperature than enzymatic hydrolysis (Castillo et al., 2000). In this study, because pH was adjusted following $CaCl_2$ addition, the observed effect of *CCAL* was due to the effect of $CaCl_2$ on aggregation. This suggests that the large effect of temperature on aggregation at high temperatures (large Q_{10} value) was masking the smaller effect that *CCAL* would have on aggregation kinetics; it was only at low temperatures that the effect of *CCAL* becomes apparent.

Fagan et al. (2007) found that the LFV sensor response during syneresis is correlated to changes in curd moisture and whey fat content. The response surface graphs confirm those findings for ΔR_{syn} because they indicate that ΔR_{syn} tends to be small when there are high fat losses or the extent of whey release is low.

For example, ΔR_{syn} as a function of temperature was maximized at approximately 30°C (Figure 3d and e), which was within the temperature range at which *WFL* was minimized (Figure 1a and b). The decrease in ΔR_{syn} with increasing *CCAL* may be explained as the effect of increased TS retention on CY_{db} (Figure 2a). The effect of t_{cut} on ΔR_{syn} (Figure 3b) seems to be represented by the effect of t_{cut} on CY_{db} (Figure 2b), because at low temperatures (27°C), CY_{db} was minimized at approximately a t_{cut} of 2.6, whereas ΔR_{syn} was maximized at a t_{cut} of ~2.6. In Figure 3f, the lowest value of ΔR_{syn} corresponded with long t_{cut} and high *CCAL* (3.7 and 3 m*M*) at 33°C. This suggests the gel was too firm when cut, and allowed rapid coarsening and microsyneresis to occur, ultimately resulting in the highest *WFL* (Figure 1c). High *CCAL* (3 m*M*) and short t_{cut} (1.8) tended to maximize ΔR_{syn} (Figure 3f), which was also attributed to the high retention of TS and fat (Figure 2c and f). These results suggest that the LFV sensor was sensitive to changes in moisture and TS retention, particularly fat.

CONCLUSIONS

The effects of temperature, calcium chloride addition level, and cutting time on curd moisture content, whey fat losses, and curd yield were examined. These results support the existence of an optimum level of curd firmness at cutting. The *WFL* was predominantly affected by temperature and t_{cut}. Temperature was also found to affect the rate of syneresis. Temperature, *CCAL*, and t_{cut} significantly affected curd yield. It was concluded that there is an optimum firmness at which the gel should be cut to achieve maximum retention of fat as well as an optimum curd moisture content that will maximize product yield and quality. The *CFR* and CY_{db} were maximized in the temperature range 29 to 35°C depending on the *CCAL* and t_{cut} used. Below this temperature range, the gel was fragile when cut, which can result in a fine curd and high fat loss. Above this temperature range, the network becomes more rigid, rapid coarsening occurs, and the gel has greater porosity, all of which assist in the release of fat. It was concluded that within an optimal temperature range, possibly 28 to 35°C, a shorter t_{cut} and higher *CCAL* will maximize the retention of TS, casein, and fat, but reduce curd moisture content. A longer t_{cut} and lower *CCAL* will lower retention of TS but increase curd moisture. Therefore, to maximize curd yield and quality it is necessary to maximize firmness while avoiding microsyneresis. Optical parameters derived from the light backscatter profile of the LFV sensor during coagulation were found to be a function of temperature and *CCAL*, whereas the parameters generated from the LFV sensor profile during syneresis were found to be a function of temperature and t_{cut}. These results contribute to the optimization of the cheese-making process and confirm the great potential of a light backscatter sensor with a large field of view as an online sensor technology that could provide comprehensive control of the cheese-making process within the vat.

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