

# Effect of temperature and inoculum concentration on gel microstructure, permeability and syneresis kinetics. Cottage cheese-type gels

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Received 5 January 2004; accepted 24 February 2005

## Abstract

Effects of coagulation temperature and inoculum concentration on microstructure, permeability and syneresis of cottage cheese gels were investigated. Relationships between coagulation parameters (obtained by rheology and near infrared light backscatter) and rate and extent of syneresis were studied. Mass of drained whey was fitted to first order equation  $W = W_{\infty}(1 - e^{-k_{\text{whey}}t})$  (determination coefficient,  $R^2 = 0.994 \pm 0.005$ ). Temperature coefficient and activation energy for syneresis were estimated. Increasing temperature significantly enhanced the rate of syneresis, due to bond relaxation. Increasing inoculum concentration decreased rate of syneresis, suggesting that faster acidification rate inhibited network rearrangement during whey expulsion. Syneresis parameters were highly correlated with the rates of acidification and network formation and with the loss tangent. Permeability coefficient, mass of whey drained and kinetic rate constant for syneresis were predicted by equations that included temperature and coagulation parameters. The close interactions between coagulation and syneresis kinetics suggested that it may be possible to develop optical sensors to simultaneously monitor both of these processes.

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**Keywords:** Cottage cheese; Milk gels; Light backscatter; Microstructure; Syneresis; Permeability coefficient

## 1. Introduction

One of the objectives of cheese making is to obtain a curd with a defined moisture content. In fact, variations in cheese making procedures very frequently relate to different methods of controlling curd syneresis in order to obtain the desired moisture, acidity, and texture of the product. For this reason, syneresis is considered to be one of the most important steps in cheese making (Walstra, 1993). Rearrangement of casein micelles during the syneresis process is responsible for the shrinkage of the casein matrix and subsequent expulsion of whey from curd pieces. The rate and extent of

syneresis controls the moisture and lactose contents of curd (Weber, 1989; Castillo, 2001), which impacts cheese moisture and pH, and consequently cheese texture, colour, flavour and quality. Syneresis also influences protein and fat losses in whey and thus cheese yield. Better control of the syneresis process would result in an improvement of the final cheese product homogeneity and quality.

Syneresis process and factors affecting its extent and kinetics have been widely studied in rennet-induced milk gels (Walstra, 1993), but very little is known about syneresis in acid gels or in milk gels made by the combined action of acid and rennet (Lucey, 2001); we will use the term mixed gels for the gels made by a combination of acid and rennet. Limited knowledge is available about the mechanisms by which the microstructure and rheological properties of mixed gels

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influence gel porosity, permeability and endogenous syneresis pressure. Despite the importance of the syneresis process, it constitutes one of the least understood phases of cheese making, especially in mixed gels, such as cottage cheese.

Rate and extent of syneresis depends on the equilibrium between the pressure gradient within the gel network and the resistance to whey expulsion (e.g., permeability) (Walstra, Van Dijk, & Geurts, 1985). Important relationships between the syneresis reaction and coagulation factors and/or rheological properties of gel have been reported (Marshall, 1982; Weber, 1989; Van Vliet, Van Dijk, Zoon, & Walstra, 1991; Kaytanli, Erdem, & Tamer, 1993; Lucey, 2001; Walstra, Geurts, Noomen, Jellema, & Van Boekel, 2001).

Empirical syneresis techniques have been used to: (a) measure curd shrinkage by the height, volume or mass; (b) measure whey expulsion or the degree of dilution of an added tracer; (c) determine dry matter content of the curd pieces; and (d) determine curd grain density. Few authors have directly measured curd shrinkage because of measurement difficulties (Walstra et al., 1985). Van Dijk (1982) and Van Dijk and Walstra (1986) found a power law dependence between the one-dimensional shrinkage ( $\Delta H$ ) of curd slabs, studied by a microscope, and time with the exponent ranging from 0.5 to 0.78. Lodaite, Ostergren, Paulsson, and Dejmek (2000) proposed the use of a laser displacement sensor to monitor the shrinkage of curd slabs. They found that the time exponent ranged from 0.8 to 1.2. By contrast with one-dimensional curd shrinkage, the amount of whey expelled from the curd grains can be easily measured. Most research on kinetics of syneresis has been performed by direct or indirect measurement of the whey mass or volume expelled from curd grains. Marshall (1982) used a first-order kinetic equation to describe the rate of syneresis:

$$\text{Log}(C_0/C) = (0.002t/2.303) + 0.023, \quad (1)$$

where  $C_0$  was the original milk volume and  $C$  was the curd volume after time  $t$ . Peri, Lucisano, and Donati (1985) modified this equation substituting the initial milk volume ( $C_0$ ) with the final whey volume drained at infinite time ( $V_\infty$ ) as follows:

$$(V_\infty - V_t) = V_\infty e^{-kt}, \quad (2)$$

where  $V_t$  was the amount of whey released at time  $t$  and  $k$  was the kinetic constant for syneresis. Peri et al. (1985) found that Eq. (2) was in good agreement with experimental results although Dejmek and Walstra (2004) considered that the good fit might have been due to this equation having two adjustable parameters. Other authors (Noel, Ramet, Gervais, Lablée, & Cerf, 1989; Kaytanli et al., 1993; Castillo, Jordán, Godoy, Laencina, & López, 2000a) also claimed that syneresis was described by first-order kinetics. There does not

appear to be any published report on the syneresis kinetics of mixed gels. The objectives of this study were to: (a) determine the effect of inoculum concentration and coagulation temperature on the kinetics of syneresis and permeability coefficient of mixed milk gels; (b) determine if there are relationships between gel formation properties and syneresis behaviour; and (c) determine if parameters characterizing gel formation can be used to predict the parameters characterizing syneresis.

## 2. Materials and methods

Data analyzed in this study correspond to the data set presented by Castillo, Lucey, and Payne (2005a). A brief description of materials and methods aspects with particular relevance is provided here. For further details, the reader should refer to the previous paper. A randomized factorial design with three replications was performed. Three levels of starter culture (0.5, 2.75 and 5% (w/w)) and three gelation temperatures (22, 27 and 32 °C) were investigated using a constant concentration of  $\text{CaCl}_2$  and chymosin. Coagulation was monitored, until pH 4.8, using a light (880 nm) backscatter sensor, a rheometer and a pH-meter. Each test day, a 3 L sample of reconstituted low heat skim milk powder at the target temperature was inoculated at the target level of starter culture, as shown in Fig. 1. Light backscatter measurements in the reaction vessel were taken simultaneously with the starter addition to the milk. The milk in the flask was split into six aliquots for the rheological, pH, curd shrinkage, permeability coefficient, and whey drainage measurements and for microscopy analysis. Light backscatter was monitored as described by Castillo et al. (2005a). These authors also provided

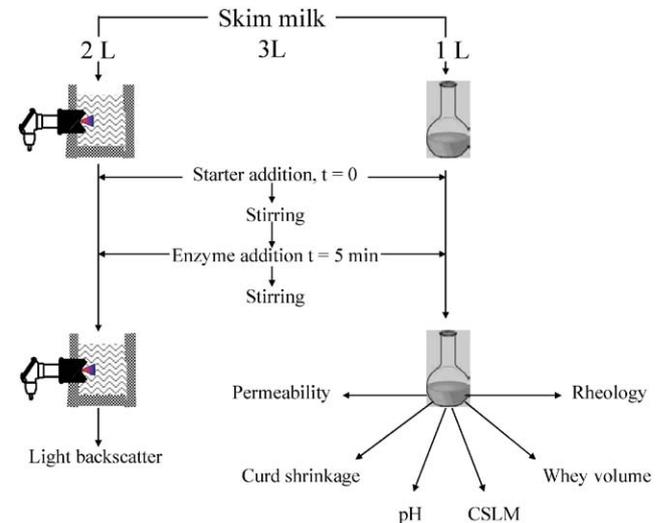


Fig. 1. The parallel experimental design utilized to compare and correlate the different techniques used in the research. CSLM, confocal scanning laser microscopy.

definitions of the light backscatter parameters used that were classified according to Castillo, Payne, Hicks, and López (2000b). Dynamic rheology testing methodology, pH measurements and the corresponding parameter definitions were as described by Castillo et al. (2005a). The recommended curd cutting time when rennet is added at  $2 \mu\text{L L}^{-1}$  is when the curd reaches pH 4.8 (Walstra et al., 2001). For that reason, the time at which the curd achieved a pH of 4.8 was selected as cutting time ( $t_{\text{cut}}^*$ ). For further details regarding cutting time see Castillo, Payne, Wang, and Lucey (2005b).

### 2.1. Permeability coefficient

Permeability coefficient of gels was measured using the method described by Van Dijk and Walstra (1986) and Roefs, de Groot-Mostert, and Van Vliet (1990) and calculated by the equation

$$B = - \left[ \ln \frac{(h_{\infty} - h_{t2})}{(h_{\infty} - h_{t1})} \right] \eta H / [\rho g (t_2 - t_1)], \quad (3)$$

where  $B$  was the permeability coefficient ( $\text{m}^2$ ),  $h_{\infty}$  was the height of the whey in the reference tube (m),  $h_{t1}$  was the height of the whey in the gel tube (m) at time  $t_1$ ,  $h_{t2}$  was the height of the whey in the gel tube (m) at time  $t_2$ ,  $\eta$  was the viscosity of the whey (Pa s),  $H$  was the length of the gel (m),  $\rho$  was the density of the whey ( $\text{kg m}^{-3}$ ) and  $g$  was the acceleration due to gravity ( $\text{m s}^{-2}$ ). Gels were formed at target temperature inside hollow glass tubes (4 mm internal diameter and 25 cm length). Permeability measurements were made at a temperature of  $30^\circ\text{C}$  and pH of 4.8. The height of whey in the tubes was measured at 10 min intervals over 2 h using a cathetometer (Model 2202, The Precision Tool and Instrument Company, East Sussex, UK) having an accuracy of 0.01 mm. The  $B$  value that was obtained was the average of 12 determinations per tube and 7 tubes per test. Samples were not included in the analysis if the gel appeared cracked or separated from the sides of the glass tubes. The viscosity of the acid whey at  $30^\circ\text{C}$  was assumed to be 0.95 mPa s (Lucey, Teo, Munro, & Singh, 1998c).

### 2.2. Degree of whey separation

The method proposed by Lucey, Munro, and Singh (1998a) was used to measure the degree of whey separation. Gels samples were tested using two 250 mL volumetric flasks that were filled to a height just below the flask neck with 240 g of milk. The flasks were maintained at the target temperature by placing them in a water bath. One flask was examined 2 h after the pH reached 4.8 to determine if whey had collected on the top or around the sides of the gel. The free whey was gently poured off for 1 min and weighed. The variable  $W_{2h}$  was defined as the relative mass of whey collected

from the undisturbed gel samples 2 h after they reached pH 4.8.  $W_{2h}$  was expressed as a percentage of the initial weight of milk. The second flask was used to estimate the syneresis kinetics by decanting and weighing the free whey every 10 min intervals for 2 h after the gel reached pH 4.8. The expulsion of whey from the mixed gel was assumed to follow a first order kinetics as has been observed for rennet-induced milk gels (Marshall, 1982; Peri et al., 1985; Weber, 1989; St-Gelais & Haché, 1995; Grundelius, Lodaite, Ostergren, Paulsson, & Dejmeck, 2000; Castillo et al., 2000a). The following first order equation was fitted to the data:

$$W = W_{\infty}(1 - e^{-k_{\text{whey}}t}), \quad (4)$$

where  $W$  was the weight of whey (g) at time  $t$  (min),  $W_{\infty}$  was the mass of whey drained (g) at infinite time (estimated to be 120.3 g), and  $k_{\text{whey}}$  was the kinetic rate constant ( $\text{min}^{-1}$ ) for the syneresis process.

The variable  $W_A$  was the accumulated whey mass collected every 10 min during the 2 h period after pH 4.8 and was expressed as a percentage of the initial weight of milk.

### 2.3. One-dimensional curd shrinkage

One-dimensional shrinkage of the gels was measured using the method described by Lodaite et al. (2000). A laser (LB-72W, Keyence Corporation, Osaka, Japan) connected to an analog sensor controller (RD-50RW, Keyence Corporation) measured the displacement of the gel surface during syneresis. Measurements were taken at 60 ms intervals. The displacement resolution was  $2 \mu\text{m}$  at the response speed selected. The laser system was calibrated by placing the sensor head at different distances from the target surface. Center of measuring range (0 V output) was set at 40 mm from the target (zero point adjustment). After span adjustment, a linear regression between the output voltage and distance (in the range 30–50 mm) was conducted in order to convert the output voltage units to length units. The 122 g milk samples were placed in a temperature-controlled vessel (10.2 cm of internal diameter and 1.59 cm depth) located in the middle of the vat, which was connected to a water bath. The samples were covered with a lid to avoid dehydration of the gel during coagulation. The water bath lid had a central opening through which the laser was placed to allow distance measurements to be taken during syneresis process. The kinetics of one-dimensional curd shrinkage was estimated from the laser displacement measurements. Measurements were started when the pH of the gel was 4.8. Shrinkage was started ( $t = 0$ ) by carefully wetting the surface of the curd with 10 mL of UF milk permeate previously warmed for 10 min at the target temperature. Measurements were recorded every 10 min for a period of 2 h. Milk permeate was obtained by ultrafiltration of commercial skim milk

(molecular mass cut-off of 10,000 Da). Two different models were used to characterize the displacement of the curd surface. First, curd surface displacement data were fitted to the following first order kinetic model, based on the model proposed by Weber (1989), to obtain the rate constant of gel shrinkage:

$$H = H_{\infty} + (H_0 - H_{\infty})e^{-k_{\text{skg}}t}, \quad (5)$$

where  $H$  was the curd height (mm) at time  $t$  (min),  $H_{\infty}$  was the height of the curd slab (mm) at infinite time (estimated to be 13.04 mm),  $H_0$  was the initial curd height (mm), and  $k_{\text{skg}}$  was the one-dimensional gel shrinkage kinetic rate constant ( $\text{min}^{-1}$ ). Secondly, the curd surface displacement data were fitted to a power law equation as proposed by Van Dijk and Walstra (1986) and by Lodaite et al. (2000):

$$\Delta H = at^b, \quad (6)$$

where  $\Delta H$  was the change in the surface position (mm),  $a$  and  $b$  were constants, and  $t$  was time (min).

#### 2.4. Confocal scanning laser microscopy (CSLM)

The CSLM technique was used for evaluating the microstructure of acid milk gels (Lucey, Tamehana, Singh, & Munro, 1998b). A fluorescent protein dye (Fast Green FCF, Merck, Darmstadt, Germany) was dissolved in demineralized water (~0.2%, w/w) and approximately 300  $\mu\text{L}$  added to 50 g of milk. After the addition of culture, the milk sample was stirred for ~1 min and then a few drops of the mixture was transferred to special object glasses having a cavity and a coverslip was placed over the sample. The object glass was placed in a petri dish and held at the coagulation temperature in an incubator having a temperature control accuracy of  $\pm 0.1$  °C. The gels were examined using a confocal microscope (Bio-Rad MRC 1024, Bio-Rad Laboratories, CA, USA) with a 100  $\times$  oil immersion objective (numerical aperture = 1.4). CSLM was used with an excitation wavelength of 568 nm. All experiments were performed in duplicate, many fields viewed and typical micrographs reported.

#### 2.5. Statistical analysis

The data were analyzed using the Statistical Analysis System (SAS<sup>®</sup>, 1999) as described by Castillo et al. (2005a) and Castillo et al. (2005b).

### 3. Results and discussion

The experimental design facilitated a comparison between whey drainage and one-dimensional curd shrinkage kinetic rate constants and helped investigate the correlations between coagulation and syneresis

Table 1  
Analysis of variance and  $F$  statistics for syneresis dependent variables<sup>a</sup>

Model	Variation source							
			$T$		$S_0$		$S_0 \times T$	
	$R^2$	$F$	DF	$F$	DF	$F$	DF	$F$
$W_{2h}$	0.986	117.9***	2	515***	2	47.3***	4	12.1***
$W_A$	0.928	20.5***	2	89.8***	2	7.63**	4	2.32 <sup>ns</sup>
$k_{\text{whey}}$	0.904	15.1***	2	61.7***	2	7.33**	4	3.10*
$k_{\text{skg}}$	0.766	5.23**	2	16.9***	2	1.42 <sup>ns</sup>	4	1.29 <sup>ns</sup>
$B$	0.896	12.97***	2	57.9***	2	2.15 <sup>ns</sup>	4	0.92 <sup>ns</sup>

<sup>a</sup>Number of observations,  $N = 27$ ;  $T$ , temperature;  $S_0$ , starter concentration;  $S_0 \times T$ , starter  $\times$  temperature interaction;  $R^2$ , determination coefficient;  $F$ , ANOVA  $F$ -statistic; DF, degree of freedom; \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , <sup>ns</sup> not significant; dependent variables explained in the text.

processes (Fig. 1). An analysis of variance (ANOVA) was conducted to determine the main sources of variation in the dependent variables and the  $R^2$  values and  $F$ -statistics were reported for each syneresis dependent variable (Table 1). The preliminary ANOVA model consisted of main effects starter concentration “ $S_0$ ”, temperature “ $T$ ” and “ $Rep$ ” (to quantify the effect of replication) and the interaction between  $T \times S_0$ . Replication effect was kept in the final model since it was found to be significant for the one-dimensional curd shrinkage kinetic rate constant,  $k_{\text{skg}}$ . The ANOVA model was found to be highly significant for all the syneresis parameters studied ( $P < 0.01$ ). Details about the ANOVA of other parameters mentioned here were discussed in detail by Castillo et al. (2005a).

#### 3.1. Kinetics of syneresis

The mass of whey expelled from the gel at 10 min intervals was fitted to Eq. (4). The  $R^2$  value and SEP for the regression between the observed and predicted data were in the range  $0.994 \pm 0.005$  and  $3.097 \pm 1.383$  g, respectively. This indicated that whey expulsion from mixed milk gels followed first order kinetics. Fig. 2 shows an example of the fitting of Eq. (4) to experimental data for milk coagulated at 32 °C with the addition of 2.75% of starter. The kinetic rate constants for whey drainage,  $k_{\text{whey}}$ , for the 27 tests were in the range  $5.07 \times 10^{-3} \pm 2.46 \times 10^{-3} \text{ min}^{-1}$ . These values were either on the same order of magnitude or one order of magnitude smaller (depending on the conditions) than the values reported by Calvo and Balcones (2000), one order of magnitude smaller than the values reported by Kaytanli et al. (1993) and Castillo et al. (2000a) and two orders of magnitude smaller than values published by Peri et al. (1985). This was as expected since these previous reports estimated the rate constant for rennet-induced milk gels, which have been reported to have a larger loss tangent (which indicates

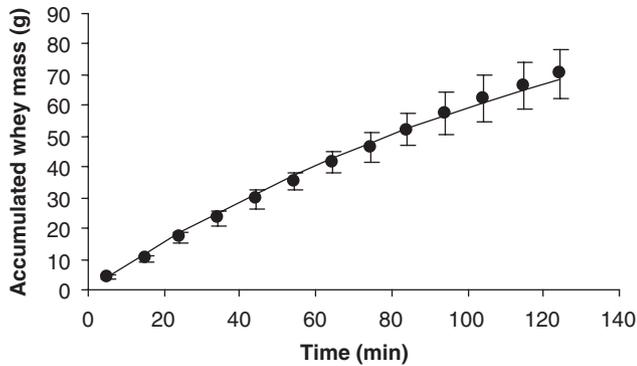


Fig. 2. Kinetics of whey expulsion as a function of time. Time zero corresponds to the time at which the gel pH reached a value of 4.8. Initial mass of milk was 240 g. Skim milk (10% total solids) coagulated at 32 °C with addition of chymosin (2 mg kg<sup>-1</sup>), calcium chloride (0.2 g kg<sup>-1</sup>) and starter (2.75% w/w). (—) Theoretical curve corresponded to 32 °C and 2.75% assuming a first order kinetic reaction for syneresis process (Eq. (4):  $W_{\infty} = 120.3$  g;  $k_{\text{whey}} = 6.83 \times 10^{-3}$  min<sup>-1</sup>;  $R^2 = 0.996$ ; SEP = 1.76 g). (●) Average value of the experimental data at the test conditions ( $N = 3$ ). Error bars corresponded to the  $\pm 1$  SD interval with the average value being the middle point.

that the network may undergo greater rearrangements), permeability coefficient, endogenous pressure and initial rate of syneresis than acid gels (Van Vliet et al., 1991). No reports were found for the kinetic rate constant for syneresis in acid-type gels or mixed gels. It was found that  $k_{\text{whey}}$  was a quadratic function of temperature. The temperature coefficient ( $Q_{10}$ ) for the drainage of whey reaction was calculated to be 2.6 for mixed gels. Kaytanli et al. (1993) reported a  $Q_{10}$  value of 1.8 in rennet-induced milk gels. The Arrhenius plot for  $\ln(k_{\text{whey}})$  against  $1/T$  (K<sup>-1</sup>) was used to estimate the activation energy ( $E_a$ ) for the drainage of whey.  $R^2$  for these regressions were 0.913, 0.974 and 0.853 for starter concentrations of 0.5, 2.75 and 5%, respectively.  $E_a$  was estimated to be 82.5, 72.8 and 57.6 kJ mol<sup>-1</sup> for starter concentrations of 0.5, 2.75 and 5%, respectively.  $E_a$  appeared to decrease with increasing inoculation levels and this decrease was statistically significant between the two extreme starter concentrations ( $P < 0.05$ ).

The one-dimensional displacement of gel surface was measured simultaneously with the expulsion of whey. One-dimensional curd displacement data were fitted to Eqs. (5) and (6). The  $R^2$  value for the regression between the observed and predicted data points (Eq. (5)) for the 27 tests was in the range  $0.917 \pm 0.159$ . Note that the estimated syneresis constants obtained by measurement of whey release ( $k_{\text{whey}}$ , Eq. (4)) or by measurement of one-dimensional curd shrinkage ( $k_{\text{skg}}$ , Eq. (5)) had the same order of magnitude, even though  $k_{\text{skg}}$  values were slightly larger than  $k_{\text{whey}}$  values when compared at the same temperature or at the same starter level (Table 2). The  $Q_{10}$  was 2.4 when calculated by using  $k_{\text{skg}}$  and was reasonably similar to the  $Q_{10}$  of 2.6 calculated by using  $k_{\text{whey}}$ . The low  $R^2$  values obtained by fitting experi-

Table 2

Influence of main effects (temperature and starter concentration) on the dependent variables for syneresis and permeability coefficient<sup>a,b</sup>

	Main effects					
	Temperature <sup>c</sup> (°C)			Starter concentration <sup>d</sup> (%)		
	22	27	32	0.5	2.75	5
$W_{2h}$ (%)	0.990 <sup>a</sup>	2.39 <sup>b</sup>	8.96 <sup>c</sup>	5.58 <sup>a</sup>	3.60 <sup>b</sup>	3.15 <sup>b</sup>
$W_A$ (g)	15.6 <sup>a</sup>	20.9 <sup>b</sup>	32.3 <sup>c</sup>	25.8 <sup>a</sup>	21.4 <sup>b</sup>	21.6 <sup>b</sup>
$k_{\text{whey}}$ (10 <sup>-3</sup> min <sup>-1</sup> )	3.01 <sup>a</sup>	4.29 <sup>b</sup>	7.91 <sup>c</sup>	6.08 <sup>a</sup>	4.57 <sup>b</sup>	4.56 <sup>b</sup>
$k_{\text{skg}}$ (10 <sup>-3</sup> min <sup>-1</sup> )	5.03 <sup>a</sup>	6.25 <sup>a</sup>	12.2 <sup>b</sup>	7.89 <sup>a</sup>	8.92 <sup>a</sup>	6.69 <sup>a</sup>
$B$ (10 <sup>-13</sup> m <sup>2</sup> )	2.21 <sup>a</sup>	3.06 <sup>b</sup>	4.17 <sup>c</sup>	3.35 <sup>a</sup>	2.98 <sup>a</sup>	3.11 <sup>a</sup>

<sup>a</sup>Least squares means (LSM) with same letters are not significantly different ( $P < 0.05$ ); number of replications = 3;  $N = 27$ .

<sup>b</sup>For dependent variables definition, see the materials and methods section.

<sup>c</sup>LSM for each temperature was based on average of nine trials over a range of three starter concentration levels.

<sup>d</sup>LSM for each starter concentration was based on average of nine trials over a range of three temperature levels.

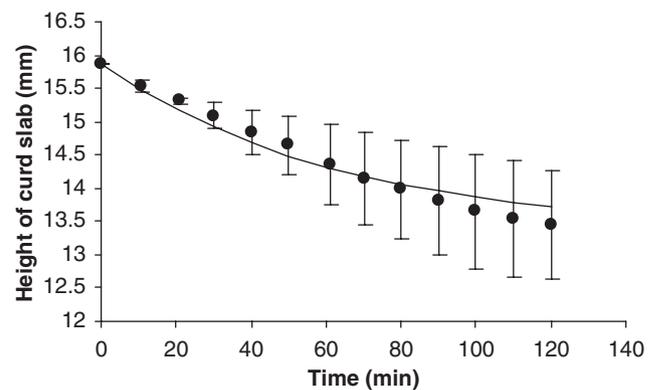


Fig. 3. Kinetics of one-dimensional shrinkage of a curd slab during syneresis process. Time zero corresponds to the time at which the gel pH reached 4.8. Initial height of curd slab equal to 15.9 mm. Skim milk (10% total solids) coagulated at 32 °C with addition of chymosin (2 mg kg<sup>-1</sup>), calcium chloride (0.2 g kg<sup>-1</sup>) and starter (0.5% w/w). (—) Theoretical curve corresponds to 32 °C and 0.5% starter concentration assuming a first order kinetic reaction for decrease of the curd slab height during syneresis process (Eq. (5):  $H_{\infty} = 13.0$  mm;  $k_{\text{skg}} = 1.4 \times 10^{-2}$  min<sup>-1</sup>, average  $R^2 = 0.963$ ; average SEP = 0.23 mm). (●) Average value of the experimental data at test conditions ( $N = 3$ ). Error bars corresponded to the  $\pm 1$  SD interval with the average value being the middle point.

mental one-dimensional syneresis data points to Eq. (5) suggested that measurement of one-dimensional gel surface displacement could be less adequate for a description of the syneresis phenomenon than measurement of whey drainage. As compared with Eq. (5), Eq. (6) had a poorer ability to describe the shrinkage of curd ( $R^2 = 0.599 \pm 0.292$ ). The low  $R^2$  observed for Eqs. (5) and (6) were attributed to the large standard deviation of this measurement method. The variability observed in our experimental data (Fig. 3) may have resulted from

Table 3

Determination coefficients for the fit of the experimental data to the equations describing whey separation (Eq. (4)) and one-dimensional curd shrinkage (Eqs. (5) and (6)) during curd syneresis<sup>a</sup>

	R <sup>2</sup> values		
	Model		
	Eq. (4) $W = W_{\infty}(1 - e^{-k_{\text{whey}}t})$	Eq. (5) $H = H_{\infty} + (H_0 - H_{\infty})e^{-k_{\text{skg}}t}$	Eq. (6) $\Delta H = at^b$
Temperature <sup>b</sup> (°C)			
22	0.9940	0.9471	0.4320
27	0.9936	0.8670	0.5381
32	0.9941	0.9357	0.8266
Starter concentration <sup>c</sup> (%)			
0.5	0.9960	0.8438	0.4670
2.75	0.9940	0.9795	0.6415
5	0.9918	0.9265	0.6883

<sup>a</sup>Number of replications = 3;  $N = 27$ .

<sup>b</sup>R<sup>2</sup> for each equation and temperature was based on average of nine trials over a range of three starter concentration levels.

<sup>c</sup>R<sup>2</sup> for each equation and starter concentration was based on average of nine trials over a range of three temperature levels.

the wetting procedure. Lodaite et al. (2000) reported that the wetting procedure had a large impact on the curd shrinkage measured by a laser displacement sensor. They attributed this effect to the deformation of the surface. Using Eq. (6) we observed a time exponent in the range  $1.534 \pm 0.885$ , which was slightly larger than that observed by Van Dijk and Walstra (1986) (0.5–0.78) and closer to the values observed by Lodaite et al. (2000) (0.8–1.2, for the first 200 s of syneresis). Differences in the time exponent may be a result of the thickness of the curd used and/or the syneresis interval studied ( $t = 2$  h). In summary, Table 3 compares the capability of Eqs. (4–6) to describe the experimental data corresponding to whey separation or one-dimensional curd shrinkage at different temperatures and starter concentrations. Overall, the results suggested that a first order model (Eqs. (4) and (5)) was the most appropriate model for describing syneresis in mixed milk gels.

### 3.2. Influence of temperature on syneresis and permeability coefficient

The average  $B$  value obtained for all mixed gels,  $0.319 \pm 0.090 \mu\text{m}^2$ , is in agreement with that for rennet and acid gels (Lagoueyte, Lablee, Lagaude, & Tarodo de la Fuente, 1994; Lucey, Van Vliet, Grolle, Geurts, & Walstra, 1997). Table 2 shows the least squares means (LSM) for the parameters characterizing the syneresis rate ( $k_{\text{whey}}$ ,  $k_{\text{skg}}$ ,  $W_{2\text{h}}$ ,  $W_A$ ) and permeability coefficient. The LSM values for  $k_{\text{whey}}$ ,  $k_{\text{skg}}$ ,  $W_{2\text{h}}$ ,  $W_A$ , and  $B$  increased significantly ( $P < 0.05$ ) with increasing gelation temperature. In mixed gels, the higher the temperature,

the higher the permeability, the faster the syneresis reaction and the larger the amount of whey separation. Van Vliet et al. (1991) reported that, in rennet gels, an increase in the coagulation temperature in the range 20–35 °C raised the parameters studied ( $B$ ,  $\text{d}B/\text{d}t$ , endogenous syneresis pressure, and one-dimensional shrinkage after 5 min). Similar behaviour in rennet gels as a function of temperature has been reported by other authors (Van Dijk & Walstra, 1986). Regarding acid and mixed gels, the literature is very limited. Lucey et al. (1998b) found, in acid skim milk gels made with bacterial culture or with glucono- $\delta$ -lactone, that increasing incubation temperature increased  $B$  and whey separation in the range 30–42 °C. Confocal scanning laser micrographs of mixed gels made at 22 and 32 °C using bacterial culture are shown in Fig. 4. Independently of the starter culture concentration, gels made at 22 °C (Fig. 4a and b) appeared to be more homogeneous with a more branched and interlinked structure, while gels made at 32 °C (Fig. 4c and d) had a coarser microstructure having much less cross-linking, and exhibiting larger pores, which was in agreement with the higher  $B$  values and the faster syneresis observed at higher temperatures. Chymosin is much more active at 32 °C compared with 22 °C so it is possible that there was more proteolysis at the higher temperature. Additional proteolysis could assist in the reduction in the interconnectivity of the gel network and encourage greater rearrangements.

### 3.3. Effect of temperature on whey flow rate

The relationship between temperature and whey flow rate was investigated by Darcy's law. One-dimensional flow of a liquid (whey) through a porous media, e.g., in rennet gels, is governed by the Darcy's law (Van Dijk & Walstra, 1986; Lucey, 2002):

$$v = \frac{B \Delta P}{\eta \Delta x}, \quad (7)$$

where  $v$  ( $\text{m s}^{-1}$ ) is the linear flow rate of the liquid defined as  $Q/A$  ( $Q$  is volumetric flow rate in the  $x$  direction and  $A$  is the cross-sectional area of flow perpendicular to  $x$ ),  $B$  ( $\text{m}^2$ ) is the permeability coefficient for the porous media,  $\Delta P/\Delta x$  ( $\text{Pa m}^{-1}$ ) is the pressure gradient of the liquid in the direction of flow and  $\eta$  ( $\text{Pa s}$ ) is the viscosity of the flowing liquid. According to Lucey et al. (1997) and Lodaite (2002), the pressure causing whey expulsion in rennet gels is given by

$$\Delta P = P_s + P_g + P_e - P_r, \quad (8)$$

where  $P_s$  is the endogenous syneresis pressure induced by network rearrangement,  $P_g$  is the pressure on the whey caused by the matrix weight,  $P_e$  is any external mechanical pressure and  $P_r$  is the pressure due to the reaction force exerted by the compressed network. In our

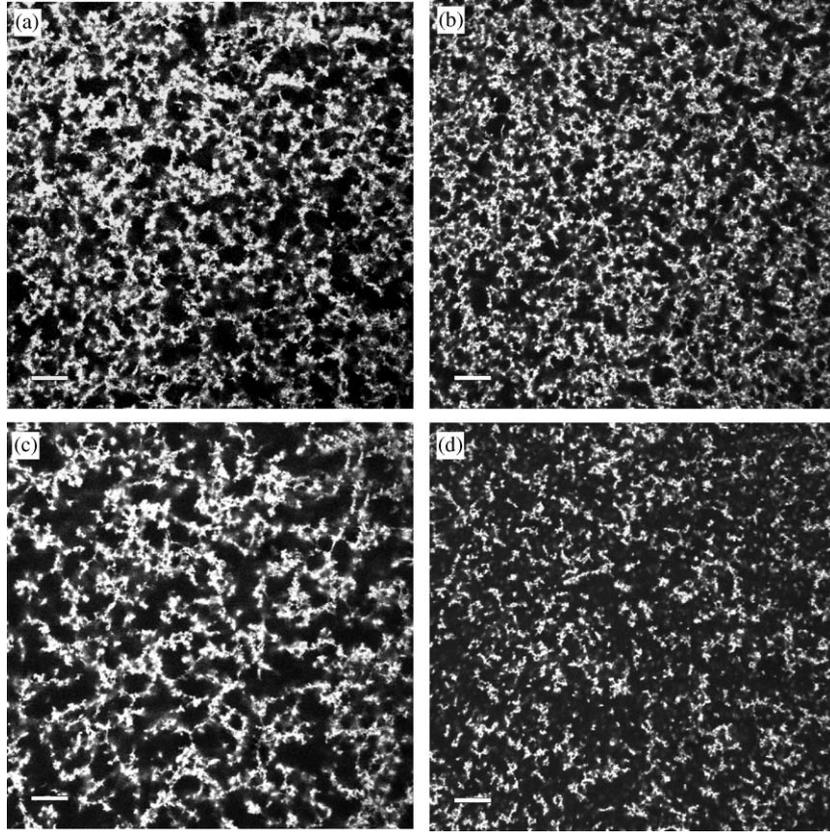


Fig. 4. Confocal scanning laser micrographs of mixed milk gels formed by acidification with (a) 0.5% (w/w) starter culture at 22 °C, (b) 5% (w/w) starter culture at 22 °C, (c) 0.5% (w/w) starter culture at 32 °C, (d) 5% (w/w) starter culture at 32 °C. Skim milk used had 10% total solids. Chymosin and calcium chloride were added to the skim milk at a concentration of 2 mg kg<sup>-1</sup> and 0.2 g kg<sup>-1</sup>, respectively. Scale bar = 20 μm. The protein network appears white while the pores appear dark.

conditions, external forces exerted were negligible and  $P_e \approx 0$ . Lucey et al. (1997) reported that in acid gels at temperatures less than 30 °C,  $P_s$  was negligible. Since, in our experimental conditions, the highest temperature was 32 °C, we assumed that  $P_s \approx 0$ . Then, for our test conditions,  $\Delta P$  was a function of  $P_g$  and  $P_r$ .  $P_g$  is given by the weight difference between the protein matrix and the surrounding whey as follows (Lucey et al., 1997):

$$P_g = x\phi_{\text{net}}g\Delta\rho, \quad (9)$$

where  $x$  (m) is the depth below the surface,  $\phi_{\text{net}}$  is the net volume fraction of casein,  $g$  (9.8 m s<sup>-2</sup>) is the acceleration due to gravity and  $\Delta\rho$  (kg m<sup>-3</sup>) the density difference between casein and liquid.  $P_r$  could be calculated by the equation (Lucey et al., 1997):

$$P_r = E\varepsilon, \quad (10)$$

where  $E$  (Pa) =  $2G'(1 + \mu)$  is the compression modulus ( $\mu$  is the Poisson ratio) and  $\varepsilon = \Delta x/x$  is the relative deformation. Since the network cannot expand sideways in our experimental conditions,  $\mu \approx 0$  and  $E \approx 2G'$ . Initially  $P_r = 0$  ( $\varepsilon = 0$ ), and  $P_g$  causes consolidation, which deforms the gel network and increases the  $\varepsilon$  value. Deformation would then induce an increasing  $P_r$  value with time. When  $P_r$  becomes equal to  $P_g$ , then syneresis stops.

$P_g$  is constant when  $\phi_{\text{net}}$  and  $\Delta\rho$  are constant. However, increasing temperature affects both  $\phi_{\text{net}}$  and  $\Delta\rho$ . Calculation of  $\Delta\rho$  as a function of temperature was performed according to Nguang, Chen, and Ozkan (2002). The  $\phi_{\text{net}}$  at different temperatures was calculated from the respective densities corresponding to protein and water at different temperatures and the protein composition of skim milk (milk reconstituted at 10% (w/w); protein content of skim milk powder was 0.29 g per g of powder). Temperature also affects  $P_r$  since  $G'$  of gel decreases with increasing temperature, which would lead to a decrease in  $P_r$  for a given  $\varepsilon$  value. Indeed,  $G'$  would decrease during syneresis, due to large scale network rearrangement (Lucey et al., 1997). Thus, model simulation required an estimate of the proportion of  $G'$  decrease in mixed gels during syneresis at different temperatures. The introduction of an empirical fitting parameter,  $i = 1, 2, \dots, n$ , allowed generating increasing fractions of the experimental LSM values for  $G'$  at different temperatures ( $G'_T$ ),  $G'_{i,T} = G'_T/i$ .

For model simulation, a differential equation was obtained from Eq. (7) as follows:

$$\frac{v\eta}{B} dx = dP. \quad (11)$$

Assuming that  $B$  and  $\nu$  do not change with depth, Eq. (11) was integrated between  $x_0 = 0$  (surface) and  $x_1$ , and between  $P_0$  and  $P_1$ , and solved for  $\nu$  yielding:

$$\nu = \frac{B \Delta P}{\eta x_1}. \quad (12)$$

Then, Eq. (12) was used to simulate  $\nu$  as function of temperature by substituting Eq. (8) in Eq. (12).  $P_g$  and  $P_r$  were calculated, as described above, by using Eqs. (9) and (10), respectively.  $B$  values used corresponded to experimental LSM values of  $B$  at the tested levels of temperature. For the change in whey viscosity with temperature,  $\eta$  values of whey reported by Lucey et al. (1998b) were used. A depth  $x_1 = 6$  mm below the surface and  $\varepsilon = 0.05$  were used to compare results of simulation at 32 °C with those reported by Lucey et al. (1997). Simulation was performed using ever-increasing values of  $i$ . Positive flow rates for the three temperatures considered were simultaneously obtained for  $i \geq 19$ . The simulated  $\nu$  value at 32 °C ( $i = 19$ ) was  $0.62 \times 10^{-8} \text{ ms}^{-1}$ , which agreed well with Lucey et al. (1997) ( $1.3 \times 10^{-8} \text{ ms}^{-1}$  for acid gels at 30 °C). The  $i$  value needed to obtain positive  $\nu$  values was larger than expected, i.e., very large reduction of  $G'$  during syneresis. This suggested that  $P_r$  was overestimated or that  $P_s$  was not negligible as temperature increased. This may result from the activity of the small amount of rennet in these mixed gels or the change in acid gel network properties at high temperatures (Lucey et al., 1997). The simulated  $\nu$  values increased with increasing temperature according to the observed increase of syneresis parameters with increasing temperature as shown in Table 2. Thus, the model predicted, the effects of temperature on whey flow rate according to Darcy's law.

Finally, the effect of different degrees of deformation,  $\varepsilon$ , and depths,  $x$ , on the simulated values was analyzed. Flow rates were simulated at  $x$  values of 1, 7.95, and 15.9 mm using the experimental average  $\varepsilon$  values (0.072, 0.090, and 0.127) observed 2 h after pH 4.8 was attained at 22, 27 and 32 °C, respectively. The results confirmed the above conclusions.

#### 3.4. Effect of inoculum concentration on syneresis and permeability coefficient

The effect of starter concentration on the syneresis parameters was inconclusive as shown in Table 2. The LSM values for  $B$  and  $k_{\text{skg}}$  did not change significantly with starter concentration.  $W_{2h}$ ,  $W_A$  and  $k_{\text{whey}}$  decreased significantly when starter concentration increased from 0.5 to 2.75%, while the differences between 2.75 and 5% levels were not significant. No definite conclusions on the effects of starter concentration on syneresis could be obtained from the data. However, the results suggested that increasing starter concentrations tended to decrease

syneresis. Differences in microstructure between gels induced with different starter concentrations were mostly small. At low incubation temperature, the casein network structure observed at higher starter concentrations (Fig. 4b) had more homogeneous and less coarse structure with smaller pores than at low starter concentration (Fig. 4a). At high incubation temperature, the enhanced chymosin activity may have reduced gel interconnectivity especially at high starter concentrations. The observed increase of syneresis with decreasing levels of starter concentration could be related to a change from a predominant acid-type gel to a more rennet-type gel structure. It could be argued that in our experimental design, the use of constant enzyme concentration and decreasing starter concentration caused a transition to a more rennet-type gel with an improved tendency for the network to rearrange and undergo syneresis. This may explain why we observed a smooth decrease in  $W_{2h}$ ,  $W_A$ ,  $k_{\text{whey}}$  with increasing starter concentration.

#### 3.5. Relationships between gel properties and syneresis process

Pearson's correlation coefficients were calculated to evaluate evidence of linear relationships between coagulation variables and parameters characterizing syneresis (Table 4). As mentioned in the materials and methods, definitions of light backscatter, rheological and pH parameters discussed here were explained by Castillo et al. (2005a). As expected, the correlations between the various syneresis parameters ( $k_{\text{whey}}$ ,  $k_{\text{skg}}$ ,  $W_{2h}$ ,  $W_A$ ,  $B$ ) was positive and highly significant. The high positive correlation ( $P < 0.001$ ) between permeability coefficient,  $B$ , and the syneresis kinetic rate constants,  $k_{\text{whey}}$  ( $r = 0.768$ ) and  $k_{\text{skg}}$  ( $r = 0.626$ ), respectively, suggested that an increase in syneresis rate was related to an increased permeability or gel porosity. A higher rate of whey syneresis ( $k_{\text{whey}}$ ,  $k_{\text{skg}}$ ) also resulted in a larger mass of whey drained from the curd ( $W_{2h}$  and  $W_A$ ), as expected. The correlation between  $k_{\text{whey}}$  and  $k_{\text{skg}}$  was significant ( $r = 0.615$ ,  $P < 0.001$ ), showing that there was consistency between these two different approaches to syneresis kinetics.

It was confirmed that faster acidification and coagulation reactions enhanced syneresis, which was supported by (Table 4): (a) the positive correlation between syneresis parameters and the rate of acidification,  $R_A$ ; (b) the positive correlation between parameters measuring the rate of increase in light backscatter ( $R'_{\text{max}}$ ,  $R''_{\text{max}}$ ,  $R''_{\text{max}2}$ ), which represented the rate of network formation, and syneresis parameters; (c) the negative correlation observed between syneresis parameters and time-parameters characterizing acidification ( $t_{RA}$ ) as well as coagulation reactions ( $t_{\text{max}}$ ,  $t_{2\text{max}}$ ,  $t_{2\text{min}}$ ,  $t_{2\text{max}2}$ ,  $t_{2\text{min}2}$ ,  $t_{\text{gel}}$  and  $t_{\text{cut}}$ ). Surprisingly, parameters describing

the time course of coagulation ( $t_{\max}$ ,  $t_{2\min2}$  and  $t_{\text{gel}}$ ) were more correlated with syneresis parameters than cutting time.

A positive correlation coefficient was found between the light backscatter response parameter  $R_{\max}$  and the syneresis variables ( $W_{2h}$ ,  $W_A$ ,  $k_{\text{whey}}$ ,  $k_{\text{skg}}$  and  $B$ ). In turn, the light backscatter ratio at cutting time (i.e., at pH

4.8),  $R_{\text{cut}}$ , seemed to have a smaller influence on syneresis when compared with  $R_{\max}$ . Regarding the rheological parameters, only  $\tan \delta$  had a significant correlation with syneresis parameters ( $W_{2h}$ ,  $W_A$ ,  $k_{\text{whey}}$  and  $B$ ). The positive coefficient observed between  $\tan \delta$  and syneresis parameters indicated that a greater rearrangement capability of the gel at pH 4.8, as indicated by the larger  $\tan \delta$  value, was related to a larger permeability coefficient and, subsequently, to a larger extent and rate of syneresis.

Table 4  
Pearson correlation between dependent variables<sup>a</sup>

	$W_{2h}$	$W_A$	$k_{\text{whey}}$	$k_{\text{skg}}$	$B$
$W_A$	0.945***	–	–	–	–
$k_{\text{whey}}$	0.945***	0.989***	–	–	–
$k_{\text{skg}}$	0.673***	0.639***	0.615***	–	–
$B$	0.874***	0.805***	0.768***	0.626***	–
$t_{\max}$	–0.423*	–0.475*	–0.411*	–0.433*	–0.556**
$t_{2\max}$	–0.415*	–0.468*	–0.405*	–0.426*	–0.546**
$t_{2\min}$	–0.428*	–0.482*	–0.418*	–0.438*	–0.560**
$t_{2\max2}$	–0.427*	–0.478*	–0.417*	–0.428*	–0.557**
$t_{2\min2}$	–0.407*	–0.459*	–0.395*	–0.422*	–0.542**
$t_{\text{gel}}$	–0.448*	–0.495**	–0.432*	–0.451*	–0.587**
$t_{\text{cut}}$	–0.388*	–0.447*	–0.384*	–0.394*	–0.525**
$R'_{\max}$	0.818***	0.825***	0.770***	0.646***	0.871***
$R'_{\max2}$	0.755***	0.787***	0.720***	0.637***	0.835***
$R'_{\max2}$	0.501**	0.585**	0.530**	0.411*	0.597**
$R_{\max}$	0.793***	0.833***	0.816***	0.468*	0.743***
$R_{\text{cut}}$	0.078 <sup>ns</sup>	0.169 <sup>ns</sup>	0.156 <sup>ns</sup>	–0.082 <sup>ns</sup>	0.0895 <sup>ns</sup>
$G'$	–0.195 <sup>ns</sup>	–0.254 <sup>ns</sup>	–0.249 <sup>ns</sup>	0.0336 <sup>ns</sup>	–0.066 <sup>ns</sup>
$\tan \delta$	0.622***	0.615***	0.550**	0.374 <sup>ns</sup>	0.655***
$\sigma_y$	–0.308 <sup>ns</sup>	–0.280 <sup>ns</sup>	–0.321 <sup>ns</sup>	–0.237 <sup>ns</sup>	–0.142 <sup>ns</sup>
$\gamma'_y$	–0.326 <sup>ns</sup>	–0.258 <sup>ns</sup>	–0.289 <sup>ns</sup>	–0.0813 <sup>ns</sup>	–0.265 <sup>ns</sup>
$\bar{R}_A$	0.380 <sup>ns</sup>	0.471*	0.414*	0.264 <sup>ns</sup>	0.441*
$t_{RA}$	–0.387*	–0.437*	–0.382*	–0.330 <sup>ns</sup>	–0.419*

<sup>a</sup> $N = 27$ ; \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$ , <sup>ns</sup>not significant; syneresis variables,  $W_{2h}$ ,  $W_A$ ,  $k_{\text{whey}}$ ,  $k_{\text{skg}}$ , and  $B$  were explained in the text while the remaining dependent variables were defined by Castillo et al. (2005a);  $t_{\text{cut}}$  was defined as the time when the gel pH reached 4.8.

3.6. Prediction of parameters characterizing the syneresis process

The dependent and independent parameters (as well as derived parameters such as differences between time variables: e.g.,  $t_{2\min} - t_{\max}$ ) were analyzed to determine if syneresis parameters could be predicted by coagulation parameters. Table 5 showed the best three-variable models for the predictions of  $W_{2h}$ ,  $W_A$ ,  $k_{\text{whey}}$ ,  $k_{\text{skg}}$  and  $B$ . All models were highly significant ( $P < 0.0001$ ). Fig. 5 showed the regression between the actual and the predicted value of the mass of whey collected for 2 h after reaching pH 4.8. Table 5 shows that, a quadratic term for temperature was significant for the five models. This correlates very well with the established effect of temperature on chemical reaction rates, i.e., reaction rates increase exponentially with temperature (e.g., Arrhenius relationship). The term  $R'_{\min2}$ , which represents the slope of change in light backscatter close to the rheologically defined gelation time, was a significant predictor for Models I, II and III ( $W_{2h}$ ,  $W_A$ ,  $k_{\text{whey}}$ , respectively). This result was not surprising since  $R'_{\min2}$  contains information about the rate of network formation. Another light backscatter parameter,  $R_{\max}$ , was significant for the prediction of  $W_A$  and  $k_{\text{whey}}$ . This

Table 5  
Models for the prediction of the parameters characterizing the syneresis kinetics<sup>a</sup>

Predictors <sup>b</sup>	Model I $W_{2h}^c$ (%)	Model II $W_A^c$ (%)	Model III $k_{\text{whey}}^c$ ( $10^{-2} \text{ min}^{-1}$ )	Model IV $k_{\text{skg}}^c$ ( $10^{-2} \text{ min}^{-1}$ )	Model V $B^c$ ( $10^{-13} \text{ m}^2$ )
Intercept	25.6**	–42.5*	–0.0192**	0.105*	$-1.04 \times 10^{-12}$ *
$T$ ( $^{\circ}\text{C}$ )	–53.4**			–0.135*	
$T^2$ ( $^{\circ}\text{C}^2$ )	29.6***	12.6***	0.00339***	0.0665*	$1.37 \times 10^{-13}$ ***
$R'_{\min}$ ( $\text{min}^{-1}$ )					
$R'_{\max2}$ ( $\text{min}^{-2}$ )					
$R'_{\min2}$ ( $\text{min}^{-2}$ )	–35107***	–67905***	–26.1***		
$t_{2\max2} - t_{\max}$					$-1.31 \times 10^{-15}$ <sup>ns</sup>
$R_{\max}$		39.1*	0.0161**		
pH at $t_{\text{gel}}$					$2.08 \times 10^{-13}$ **
$\tan \delta$				–0.0825**	
$R^2$	0.969	0.927	0.914	0.627	0.864
$F$ -value	242	97.6	81.2	12.9	46.7
Probability of $F$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

<sup>a</sup>\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , <sup>ns</sup>not significant.  
<sup>b</sup>Predictors explained in the text or in Castillo et al. (2005a).  
<sup>c</sup>Parameters characterizing the syneresis kinetics explained in the text.

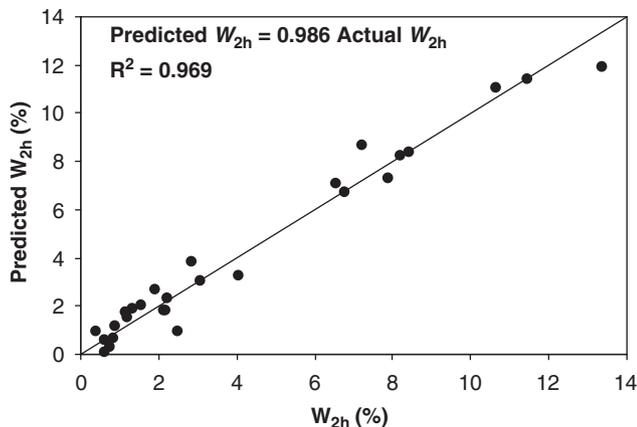


Fig. 5. Comparison of the observed and predicted mass of whey ( $W_{2h}$ ) from undisturbed gel samples 2 h after pH 4.8. Mass values expressed as a percentage of the initial mass of milk. Prediction was made using Model I (see Table 5).  $N = 27$ .  $R^2$ , determination coefficient (corrected for the means).

parameter was shown to be able to predict cutting time at constant temperature and protein concentration in rennet-induced coagulation (Castillo, Payne, Hicks, Laencina, & López, 2003). Model IV ( $k_{skg}$ ) and V(B) had smaller  $R^2$  values.

As discussed above, Eq. (4) had the highest model  $R^2$  for describing whey drainage ( $W$ ) of curd and used two parameters,  $W_\infty$  and  $k_{whey}$ . Model III estimates  $k_{whey}$  as a function  $T^2$ ,  $R''_{min2}$ ,  $R_{max}$  and an intercept. Substituting Model III for  $k_{whey}$  in Eq. (4) gives the following model for describing whey drainage over all conditions tested:

$$W = W_\infty(1 - e^{-(a+bT^2+cR''_{min2}+dR_{max})t}). \quad (13)$$

Eq. (13) was fitted to the data (27 tests) to determine  $W_\infty$  and the regression coefficients  $a$ ,  $b$ ,  $c$  and  $d$ .  $W_\infty$  was found to have a value of 164.2 g, which represented ~68% of the initial skim milk mass. Eq. (13) predicted the whey drainage ( $W$ ) as a function of time using temperature and light backscatter parameters ( $R''_{min2}$  and  $R_{max}$ ) with an  $R^2$  of 0.96 and a SEP of 4.43 g (Fig. 6). These results showed a significant interaction between coagulation kinetics and syneresis kinetics. Eq. (13) suggested that it may be possible to control the syneresis process based on measurable light backscatter parameters and temperature. Since coagulation and syneresis steps are critical for cheese yield and quality, the value of such measurements is clear.

#### 4. Conclusions

A strong correlation was observed between syneresis parameters and those parameters characterizing acid-

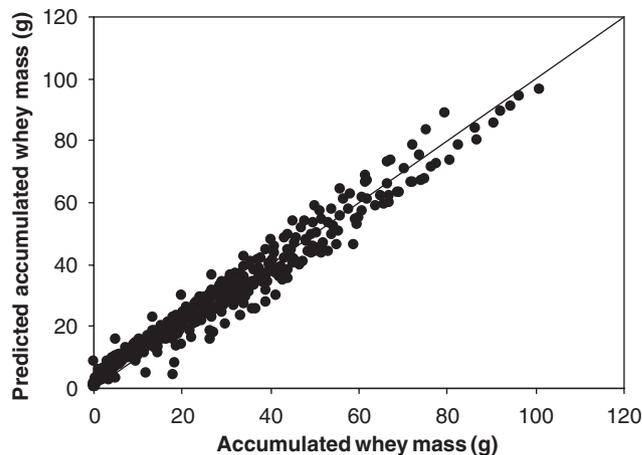


Fig. 6. Fit of Eq. (13) to the experimental values of  $W$  (accumulated whey mass at time  $t$ ) for the 27 tests, which constituted the three whole replications of the experiment. Time zero corresponded to the time at which the gel pH reached a value of 4.8. Initial mass of milk equal to 240 g. Skim milk (10% w/w total solids). Milk coagulated at 22, 27 or 32 °C, adding starter concentration of 0.5, 2.75 or 5% w/w and using constant concentration of both chymosin (2 mg kg<sup>-1</sup>) and calcium chloride (0.2 g kg<sup>-1</sup>). Parameters were:  $W_\infty = 164.2$  g;  $a = -0.0114$ ;  $b = 0.00233$ ;  $c = -17.9$ ;  $d = 0.00952$ .

ification ( $R_A$ ) and network formation ( $t_{max}$ ,  $R'_{max}$ ,  $R_{max}$ ,  $t_{gel}$ ,  $\tan \delta$ ), which showed that coagulation kinetics played an important role in the syneresis process of mixed gels. Coagulation factors, such as inoculum level and temperature, had a direct effect on the development of the casein matrix, which impacted the physical characteristic of gels (rearrangement capability, permeability coefficient). These physical characteristics affected the extent and kinetics of the syneresis process. Whey drainage in mixed gels was found to follow a first order kinetic reaction. The effect of temperature on kinetic rate constant for whey drainage allowed us to estimate both the thermal coefficient and the activation energy. The  $Q_{10}$  value for whey expulsion was ~2.6, while  $E_a$  significantly decreased from 82.5 to 57.6 kJ mol<sup>-1</sup> when the inoculum level increased from 0.50 to 5%. Increasing temperature induced a significant increase in permeability (which is related to porosity), syneresis rate and syneresis extent, while increasing inoculum level had the opposite effect. A significant interaction between the coagulation kinetics as measured by light backscatter parameters and syneresis kinetics was found. Whey expulsion with time was predicted using a model that consisted of temperature and light backscatter parameters with a coefficient of variation of 14.9%. This suggests that it may be possible to develop a sensor capable of monitoring both coagulation and syneresis process, which could lead to greater control of the moisture content and an improvement of the final cheese homogeneity and quality.

## Acknowledgements

The authors wish to thank the Wisconsin Center for Dairy Research for the use of its facilities and Reflectronics, Inc., for supplying the light backscatter measurement equipment. During this research M. Castillo was supported by a post-doctoral grant from the program MECD/Fulbright from Spanish Ministry of Education Culture and Sports financed by the Spanish Secretariat of State for Education and Universities and co-financed by the European Social Fund. This research was supported by the USDA Cooperative State Research, Education, and Extension Service (CSREES) project WIS04363.

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