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**Evaluation of a fluorescence and infrared backscatter sensor to monitor acid induced  
coagulation of skim milk.**

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**Abstract**

A prototype sensor that employs both ultraviolet excited fluorescence and infrared light backscatter was evaluated as an in-line process analytical technology (PAT) tool to monitor acid induced coagulation kinetics of skim milk. Coagulation experiments were carried out at 32 °C using three concentrations of glucono-delta-lactone (GDL). Measurement of storage modulus ( $G'$ ) of acidified skim milk gel was used as a reference rheological method to monitor the coagulation kinetics. Prediction models were developed to predict the times required for acidified skim milk coagulum to reach selected  $G'$  values (0.5 Pa, 1 Pa, 5 Pa, 10 Pa and 15 Pa) using time parameters extracted from the ultraviolet excited fluorescence and infrared light backscatter profiles. A strong correlation was observed between the predicted times developed using time parameters extracted from the prototype sensor profiles and the measured  $G'$  times extracted from the rheometer ( $R^2 = 0.97$ , standard error of prediction = 2.8 minutes). This study concluded that the prototype fluorescence and infrared backscatter sensor investigated combined with the developed rheological prediction model can be used as a potential PAT tool for in-line monitoring of coagulation kinetics in the manufacture of acid induced milk gels.

**Abbreviations:**

PAT- process analytical technology

GDL- glucono-delta-lactone

$G'$ - storage modulus

UV- ultraviolet

DAQ- data acquisition

$F$ - fluorescence response ratio

$R$ - infrared response ratio

$F'$ - first derivative of fluorescence

$R'$ - first derivative of infrared light backscatter

$F''$ - second derivative of fluorescence

$R''$ - second derivative of infrared light backscatter

$F'_{tmin}$ - time parameter corresponding to the minimum value of the first derivative of  $F$

$F'_{tmax}$ : time parameter corresponding to the maximum value of the first derivative of  $F$

$F''_{tmin}$  time parameter corresponding to minimum value of the second derivative of  $F$

$F''_{tmax}$ : time parameter corresponding to maximum value of the second derivative of  $F$

$R'_{tmin}$ - time parameter corresponding to the minimum value of the first derivative of  $R$

$R'_{tmax}$ : time parameter corresponding to the maximum value of the first derivative of  $R$

$R''_{tmin}$  time parameter corresponding to minimum value of the second derivative of  $R$

$R''_{tmax}$ : time parameter corresponding to maximum value of the second derivative of  $R$

$MT_{gel}$ - measured gel time

$PT_{gel}$ - predicted gel time

## 1. Introduction

Acid induced coagulation of milk is one of the initial steps in the manufacture of yoghurt and fresh cheese varieties i.e. quarg and cottage cheese. Different acids and acidifying agents (e.g. citric acid, lactic acid, hydrochloric acid), chemical acidulants (e.g. glucono-delta-lactone (GDL)) and bacterial cultures (fermentation of lactose to lactic acid) are selected for the acidification of milk based on the desired end-product characteristics (J. A. Lucey, 2011). Acidification of milk results in a pH reduction, promoting solubilisation of colloidal calcium phosphate from casein micelles. The micelle stability is lost and unstable casein micelles aggregate to form a network resulting in coagulum formation (Kim & Kinsella, 1989a). Previous studies have reported that different acidification methods, and factors affect the coagulation kinetics i.e. type and concentration of the acidulant used, coagulation temperature, milk composition, pre-treatment, and pH (Anema, 2008; Horne, 1999; J. Lucey, Tamehana, Singh, & Munro, 2000; J. A. Lucey & Singh, 1997; J. A. Lucey, Teo, Munro, & Singh, 1998; Phadungath, 2005). As the rate of acidification and coagulation kinetics vary with process and product parameters, it is necessary to monitor coagulation processes to improve and control final product quality characteristics.

Process analytical technology (PAT) is a framework for innovative process manufacturing and quality assurance. The goal of PAT for dairy processing is to ensure product quality through real-time measurements of critical quality and performance attributes of raw ingredients, in-process materials, processes and final products (FDA, 2004). Reported studies have investigated the development of optical, thermal and rheological sensing technologies to monitor and determine the optimal coagulum cutting time in cheese manufacture (O'Callaghan, O'Donnell, & Payne, 2002; Panikuttira, O'Shea, Tobin, Tiwari, & O'Donnell, 2018).

Fluorescence spectroscopy was used by Herbert et al. (1999) to monitor acid induced coagulation of raw bovine milk by recording and analysing the emission spectra of tryptophan in milk. The spectra obtained provided information on the changes in protein structure and interactions taking place in milk during the coagulation process. Boubellouta et al. (2011) used synchronous fluorescence and mid-infrared spectroscopy to monitor the physicochemical changes of casein micelles during acid induced coagulation of skim milk. They concluded that the spectroscopic techniques studied were complementary to rheological measurements and provide information on the changes occurring during coagulation at a molecular level. The feasibility of fluorescence measurement to predict the end point of yoghurt culture fermentation was studied by Mains et al. (2017). The data obtained from fluorescence (measured at 350 nm) was compared to the data obtained from infrared backscatter (measured at 880 nm) sensor. A linear relationship was observed between the fluorescence response and change in pH. The fluorescence response was shown to predict the endpoint of yoghurt culture fermentation with a standard error of 16 minutes and an  $R^2$  value of 0.99.

Infrared light backscatter technology has been widely employed for monitoring rennet coagulation of milk and to predict cutting time for different process and product parameters. Reported studies have shown strong correlations between predicted and measured cutting times (Arango, Trujillo, & Castillo, 2018; Castillo, Lucey, & Payne, 2006; Nicolau, Buffa, O'Callaghan, Guamis, & Castillo, 2015). Infrared measurements have also been carried out in cheese vats to monitor rennet induced coagulation kinetics of reconstituted milk and to develop cutting time prediction models (Lyndgaard, Engelsen, & van den Berg, 2012). The models developed were successfully validated in cheese trials and found to have a good statistical fit ( $R^2 = 0.99$ ).

The objectives of this study were to evaluate a prototype fluorescence and infrared backscatter sensor to monitor acid induced coagulation kinetics of skim milk and to develop rheological prediction models to determine the time required for acid induced skim milk gels to reach selected  $G'$  values (0.5 Pa, 1 Pa, 5 Pa, 10 Pa and 15 Pa).

## **2. Material and methods**

### **2.1 Skim milk:**

Skim milk (100 L) was purchased from a local supermarket and stored in a cold room maintained at 5 °C prior to experimental trials. The protein and fat levels of the skim milk were measured using a Dairy Spec FT (Bentley Instruments, Inc., Chaska, MN, USA) and were found to be 3.5 % and 0.3 % (w/w) respectively.

### **2.2 Glucono-delta-lactone (GDL):**

GDL ( ≥ 99.0 % purity, Sigma Aldrich Chemie GmbH, Kappelweg, Germany) was added at selected concentrations of 3 %, 3.5 %, 4 % (w/w) to coagulate the skim milk.

### **2.3 Acid induced coagulation:**

Acidification of skim milk was carried out in a laboratory scale cheese vat (Type CAL 10 L; Pierre Guerin Technologies, Mauze, France). Coagulating temperature was controlled using a water bath (Brookfield TC 200, Brookfield Engineering Laboratories Inc, Middleboro, Massachusetts, USA) via a heating jacket surrounding the vat. 10 kg of skim milk was added to the vat and heated to  $32 \pm 0.2$  °C with continuous stirring at 16 rpm for 45 minutes; the pH of milk was measured at 32 °C (FiveEasy pH meter Mettler-Toledo, Switzerland). After GDL addition, the milk was stirred at a speed of 31 rpm for 5 minutes. 20 ml of sample was then removed from the vat for rheological measurements. Fluorescence and infrared

backscatter profiles during acid induced coagulation of milk were acquired using a prototype sensor (Reflectronics, Lexington, KY, USA). Coagulation was monitored for 120 minutes from the point of GDL addition. The rate of pH change post addition of GDL was monitored at 20 minute intervals.

#### **2.4 Oscillatory rheology:**

The change in storage modulus ( $G'$ ) during acid induced coagulation of skim milk was monitored using a controlled stress rheometer (ARG2, TA Instruments, Crawley, UK). Tests were performed based on the method described by Everard et al. (2008) using a concentric cylindrical geometry (inner radius 15 mm and outer radius 14 mm) in oscillation mode at a constant shear strain of 0.02 % and a frequency of 1 Hz. Changes in the storage modulus  $G'$  were recorded every 30 s for 120 minutes. The times ( $MT_{gel}$ ) required to reach  $G'$  values of 0.5 Pa, 1 Pa, 5 Pa, 10 Pa and 15 Pa were recorded and rheological prediction models were developed to determine the time required for acidified skim milk gels to reach selected  $G'$  values.

#### **2.5 Fluorescence and infrared backscatter sensor:**

A prototype fluorescence and infrared backscatter sensor (FluorLite, Reflectronics, Lexington, KY, USA) was evaluated in this study. The sensor employed two optical techniques i.e. fluorescence and infrared light backscatter, to measure changes during coagulation. The UV light source in the sensor excited tryptophan in milk at 280 nm. The emitted fluorescence was measured at 350 nm. The infrared light backscatter was recorded at 880 nm. The sensor was mounted on the side wall of the vat (Figure 1) to monitor the milk coagulation kinetics. Data was acquired at 6 s intervals during the coagulation process using a data acquisition (DAQ) system (Reflectronics, Lexington, KY, USA). Fluorescence and infrared light backscatter optical profiles were recorded as the response ratios (the voltage



measurements divided by average value obtained over first minute of coagulation)  $F$  and  $R$  respectively, using the custom DAQ software. The first derivatives of fluorescence and infrared light backscatter ( $F'$  and  $R'$ ) were obtained by calculating a running slope using 10 minutes of data (100 points). The second derivatives ( $F''$  and  $R''$ ) were calculated in a similar manner. The following time parameters were obtained from the derivatives of  $F$  and  $R$ :

- $F'_{tmin}$  and  $F'_{tmax}$ : time parameters corresponding to the minimum and maximum values of the first derivative of  $F$
- $F''_{tmin}$  and  $F''_{tmax}$ : time parameters corresponding to the minimum and maximum values of the second derivative of  $F$
- $R'_{tmin}$  and  $R'_{tmax}$ : time parameters corresponding to the minimum and maximum values of the first derivative of  $R$
- $R''_{tmin}$  and  $R''_{tmax}$ : time parameters corresponding to the minimum and maximum values of the second derivative of  $R$

These time parameters were used to develop rheological prediction models to predict the time at which the acidified skim milk coagulum reached selected  $G'$  values.

### **2.6 Statistical analysis:**

The experiment was carried out in two batches, each batch included three treatments (level of GDL addition) and each treatment was carried out in triplicate ( $n = 18$ ). Reference rheological data and optical responses from the prototype sensor were statistically analysed using SAS (version 9.1; SAS Institute Inc., Cary, NC). Pearson correlation coefficients were determined by the CORR procedure. Nonlinear regression (NLIN) procedure in SAS was used to develop the rheological prediction model to predict the time at which the acidified skim milk gel reached selected  $G'$  values using the time parameters extracted from the measured optical profiles.

### 3. Results and discussion

#### **3.1 Modulation of the ionic environment (pH):**

Skim milk acidified at higher GDL concentrations resulted in milk gels at lower pH at the end of the 120 minutes incubation period (Figure 2) which is similar to the trends observed by J.A Lucey and Singh (1997), where the final pH attained in the acidified milk depended on the GDL concentration added. In the current study, a rapid decrease in pH of acidified skim milk was observed within the first 20 minutes of GDL addition followed by gradual decrease in pH during further incubation. The rapid change in pH immediately after the addition of GDL may be attributed to the rapid dissolution of GDL to form gluconic acid (J. Lucey, Van Vliet, Grolle, Geurts, & Walstra, 1997). Casein micelles are in a stable form at the natural pH of milk. The lowering of milk pH on acidification of milk causes a series of physiochemical changes in casein micelles. The net negative charge on the casein micelles is reduced as the pH of skim milk is lowered from 6.7 to 5.0 and the electrostatic repulsion between the protein molecules that is responsible for the stability of casein micelles is also reduced. The charged  $\kappa$ -casein hairs on the micelles shrink and the micelles become susceptible to aggregation. Colloidal calcium phosphates are dissolved within a pH range of 5.5 to 5.0, and the caseins are liberated into the milk serum. Aggregation of casein occurs as the pH is reduced to the isoelectric point of casein i.e. 4.6, to form a three dimensional network eventually leading to coagulum formation (Phadungath, 2005).

#### **3.2 Oscillatory rheology:**

In the current study, the onset of gel formation was defined as the time when the acidified skim milk gel reaches a  $G'$  value  $\geq 1$  Pa. In this study, it was observed that the rate of gelation was faster in skim milk acidified at higher GDL concentrations. For example, skim milk acidified with 3 % GDL reached a  $G'$  value of 1 Pa after ca. 42 minutes while skim milk

acidified with 4 % GDL reached a  $G'$  value of 1 Pa after ca. 25 minutes. At the end of the incubation period (120 minutes), it was observed that higher concentrations of GDL resulted in coagulum with higher  $G'$  values as can be seen in Figure 3.  $G'$  values of 17 and 40 Pa were recorded for skim milk acidified with 3 and 4 % GDL, respectively.

A sharp increase in  $G'$  was observed soon after the onset of gelation in skim milk acidified with 4 % GDL. The coagulum attained a maximum  $G'$  value at ca. 60 minutes after which it plateaued for the rest of the incubation period. In case of skim milk acidified with 3 % GDL concentration, the increase in  $G'$  was gradual and was observed to increase slowly till the end of the incubation period (120 minutes). This can be attributed to the accelerated dissolution of GDL and decrease in pH at higher GDL concentrations.

Horne (2003) observed that the onset of gelation was delayed for a certain period after GDL addition. The delay in the onset of gelation is due to the time required by the acidified milk to reach the gelation pH. The time at which the gelation pH is reached is influenced by the concentration of GDL added to the milk. Kim and Kinsella (1989b) observed that in skim milk acidified at 50 °C, the onset of gel formation reduced from 34 to 8 minutes on increasing the GDL concentration from 1 % to 2 % (w/v). In a similar study by Cobos et al. (1995), 2.5 % and 4 % w/v GDL concentrations were used to coagulate reconstituted whole milk and the increase in  $G'$  of acid gels formed was studied. They reported that the GDL concentration added influenced the rate of increase in coagulum  $G'$ . It was suggested by Horne (2003), that with increasing GDL concentrations, internal changes in casein micelles (loss of calcium and phosphate) and changes in localized net charge are reduced. Thus, this facilitates increased bonding in the surface regions and creates larger number of elastically active strands between the casein micelles resulting in increased storage modulus. This supports the findings of the current study, where higher storage modulus was recorded in milk

acidified at higher GDL concentrations and  $MT_{gel}$  (time required for the coagulum to reach a selected  $G'$  value) decreased at increasing concentrations of GDL addition.

### **3.3 Fluorescence and infrared backscatter response**

Typical responses from the prototype infrared light backscatter and fluorescence sensor and the change in  $G'$  measured during the coagulation process are plotted in *Figure 4*. It can be observed that the sensor had a high level of sensitivity and was capable of detecting physiochemical changes occurring in acidified skim milk before the onset of gelation ( $G' \geq 1$  Pa). The fluorescence response ratio ( $F$ ) and infrared response ratio ( $R$ ) were stable during the initial phase of acid coagulation. A sharp decrease in  $F$  was observed shortly before the onset of gelation ( $G' \geq 1$  Pa) followed by a slower decrease as the gel formation progressed. The change in fluorescence is related to pH induced physiochemical changes in casein micelle structure (Herbert et al., 1999). The fluorescence sensor used in this study excites tryptophan with UV light at 280 nm and records the fluorescence emitted by tryptophan at 350 nm. Intensity of the fluorescence recorded depends on the exposure of tryptophan in its three dimensional configuration (Herbert et al., 1999). On acidification, milk pH is lowered which leads to changes in the casein micelles as discussed in the previous section. The change in the fluorescence has been attributed to the structural and conformational changes in casein micelles due to dissociation of colloidal calcium phosphates taking place between pH 6.2 and 5.07 (Boubellouta et al., 2011). In a similar study by Mains et al. (2017), the authors concluded that the tryptophan became reoriented within the structure of casein. The reoriented tryptophan was blocked from the excitation light. This decreased the fluorescence signal received by the sensor, as the pH lowered and coagulation progressed. A similar observation was made in the current study where the fluorescence signal decreased between pH 6.0 and 5.0. In case of infrared light backscatter ( $R$ ) signal, a sigmoidal (S-

shaped) increase was observed at the initial stages of acidification followed by a stable response ratio during the gel formation phase. The change in the light backscatter was attributed to change in the molecular weight, particle size, number of colloidal casein aggregates and protein crosslinking that occur during coagulation (McMahon, Brown, & Ernstrom, 1984). In this study, infrared light backscatter measured at 880 nm was found to be most responsive during the initial stages of acid induced coagulation. In a similar study, the infrared light backscatter profiles obtained for yoghurt fermentation showed an increase in the light backscatter as the fermentation progressed. As the pH lowers from 6.0 to 5.0, the electrostatic attraction between casein micelles increase leading to aggregation, that results in an increased light backscatter (Mains et al., 2017). These findings support the observations found in the current study whereby an increase in the infrared response was recorded as the gel formation progressed.

Typical responses observed for fluorescence and infrared profiles of a typical GDL induced coagulation process showing the time parameters extracted from the optical profiles to predict the times at which the coagulum reaches selected  $G'$  values are shown in Figure 5. Table 1 shows that both the fluorescence and infrared light backscatter profiles are influenced by the acid induced coagulation kinetics at different concentrations of GDL addition. For example, the  $F'_{min}$  recorded for skim milk with 3 % and 4 % GDL addition was ca. 40 minutes and ca. 23 minutes respectively and this corresponded with the times recorded by the rheometer for the onset of gelation (i.e., ca. 42 minutes for 3 % GDL addition and ca. 25 minutes for 4 % GDL addition). All the time parameters obtained from the sensor during acid induced coagulation of skim milk were correlated with  $MT_{gel}$  for the skim milk gel to reach selected  $G'$  values (0.5 Pa, 1 Pa, 5 Pa, 10 Pa and 15 Pa). A strong correlation ( $P < 0.0001$ ) was observed between the time parameters obtained from the

prototype sensor and  $MT_{gel}$  (Table 2). It was observed that the  $MT_{gel}$  for  $G'$  values had a better correlation with the time parameters obtained from fluorescence than the time parameters obtained from infrared light backscatter. For example,  $MT_{gel}$  recorded for the coagulum to reach  $G'$  value of 15 Pa had a correlation of 0.963 with  $F'_{tmin}$ , ( $P < 0.0001$ ) giving a stronger correlation compared to the correlation with the other time parameters. The strong correlation observed between the time parameters suggested that these time parameters can be used in the development of rheological model to predict the time at which the acid induced coagulum reaches a selected  $G'$  value.

### 3.4 Development of rheological prediction model

The lack of a robust prediction model for milk coagulum  $G'$  limits the application of coagulation monitoring technologies in dairy processing. The goal of this study was to predict the time ( $PT_{gel}$ ) at which the coagulum reached a selected  $G'$  value using time parameters extracted from the prototype sensor profiles. A rheological prediction model was developed by predicting the gel firming time remaining after the occurrence of the parameter,  $F'_{tmin}$ :

$$PT_{gel} = F'_{tmin} + \text{gel firming time remaining} \text{----- Eq 1}$$

For a selected  $G'$  value, the gel firming time remaining is proportional to the GDL concentration used and time parameters extracted from the prototype sensor as expressed in the following equation:

$$\text{Gel firming time remaining} \sim (\alpha_1 * R'_{tmax} + \alpha_2 * F''_{tmin} + \alpha_3 * F''_{tmax} + \alpha_4 * GDL) \text{---} \\ \text{---- Eq 2}$$

Additionally, the prediction model is considered as a function of the selected  $G'$  value. On combining the equations 1 and 2, the following rheological prediction model was obtained:

$$PT_{gel} = F'_{tmin} + G' * (\alpha_1 * R'_{tmax} + \alpha_2 * F''_{tmin} + \alpha_3 * F''_{tmax} + \alpha_4 * GDL) \text{----Eq 3}$$

where,  $\alpha_1= 0.16$ ,  $\alpha_2= 0.11$ ,  $\alpha_3= 0.09$  and  $\alpha_4= 0.01$

This model was tested using a nonlinear regression (NLIN) procedure in SAS. The regression values illustrated that the time parameters,  $F'_{tmin}$  and  $F'_{tmax}$  did not significantly improve the model and hence were removed from the model. The resulting prediction model developed is as follows:

$$PT_{gel} = F'_{tmin} + G' * (\beta_1 * R'_{tmax} - \beta_2 * GDL) \text{----- Eq 4}$$

where,  $\beta_1 = 0.14$ ,  $\beta_2 = 0.46$

The standard error of prediction for the above prediction model is 2.8 minutes and the range error ratio (RER) is 23.26. Prediction models with RER values greater than 20 are considered to be suitable for practical application (Malley, McClure, Martin, Buckley, & McCaughey, 2005; Ward, Nielsen, & Møller, 2011). It was found that  $PT_{gel}$  obtained from the prediction model had an  $R^2$  of 0.97 with the  $MT_{gel}$  measured by the rheometer for selected  $G'$  values. Based on this level of prediction accuracy, it can be concluded that this model has the potential to be implemented for in-line determination of time at which the coagulum reaches a selected  $G'$  value in acid induced milk coagulation processes. The above model is a suggested practical model for operating in a cottage cheese or fresh cheese plant as the operator can select the required  $G'$  value based on product specific requirements. The other two constants in the model,  $\beta_1$  and  $\beta_2$ , would only change with respect to a major formulation change (e.g. change in milk composition or method of coagulation). The time-parameters used,  $F'_{tmin}$  and  $R'_{tmax}$ , are obtained from the first derivatives of  $F$  and  $R$  are considered more reliable compared to the time parameters obtained from the second derivatives.

#### 4. Conclusion

In this study, a prototype fluorescence and infrared backscatter sensor was evaluated as a PAT tool to monitor acid induced coagulation of skim milk. A rheological prediction model was developed using time parameters extracted from the sensor. The developed prediction model has the potential to be implemented for in-line process determination of the time at which the coagulum has reached a selected  $G'$  value in processes involving acid induced milk coagulation. Further validation of the developed rheological prediction models using a larger sample size is recommended to validate the robustness of the model. This study highlights the potential of using fluorescence and infrared backscatter for in-line monitoring of acid induced coagulation of milk in dairy processing applications including cottage cheese and yoghurt manufacture.

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Figure 1: Prototype fluorescence and infrared backscatter sensor and experimental set up showing prototype fluorescence and infrared backscatter sensor mounted in the side wall of the laboratory scale vat.

Figure 2: Change in pH of acidified skim milk gel at different rates of GDL addition ( $\circ$  3.00 %,  $\times$ 3.50 % and  $\square$  4.00 % w/w).

Figure 3: Change in storage modulus  $G'$  (Pa) during acid induced coagulation of skim milk at selected GDL addition rates ( $\circ$  3.00 %,  $\times$ 3.50 % and  $\square$  4.00 % w/w)

Figure 4: Change in storage modulus ( $G'$ ) measured using the rheometer and the changes in fluorescence ( $F$ ) and infrared light backscatter ( $R$ ) responses of the prototype sensor during acid induced coagulation of skim milk for GDL addition rate of 3.50 % w/w)

Figure 5: Typical responses observed from the fluorescence and infrared backscatter prototype sensor during acid induced coagulation of skim milk with the respective infrared and fluorescent time parameters.

Figure 6: Predicted gel time ( $PT_{gel}$ ) using the rheological prediction model versus measured gel time ( $MT_{gel}$ ).

Table 1: Average time parameters determined from the fluorescence and infrared backscatter profile during acid induced coagulation of milk at different concentrations of GDL.

GDL (w/w)	$F'_{tmin}$	$F''_{tmin}$	$F''_{tmax}$	$R'_{tmax}$	$R''_{tmax}$	$R''_{tmin}$
<i>Set 1:</i>						
3.0 %	39.53 ± 0.75	37.50 ± 0.80	41.13 ± 0.70	30.93 ± 0.97	21.23 ± 1.12	36.00 ± 1.59
3.5 %	29.40 ± 0.53	27.10 ± 0.46	30.93 ± 0.58	21.30 ± 1.45	15.73 ± 0.78	25.20 ± 4.19
4.0 %	22.90 ± 0.46	20.67 ± 0.38	24.47 ± 0.50	16.53 ± 0.32	12.87 ± 0.21	20.90 ± 1.50
<i>Set 2:</i>						
3.0 %	33.27 ± 2.93	29.73 ± 2.35	36.40 ± 3.41	24.67 ± 2.95	16.60 ± 3.90	32.67 ± 3.20
3.5 %	25.73 ± 0.35	22.77 ± 0.65	28.50 ± 0.96	19.23 ± 0.21	14.83 ± 0.12	26.03 ± 4.19
4.0 %	19.00 ± 0.69	15.83 ± 1.68	20.63 ± 1.55	14.47 ± 0.49	11.53 ± 0.55	16.07 ± 0.93

Table 2: Pearson correlation coefficients between the time parameters obtained and  $MT_{gel}$  recorded for selected  $G'$  values ( $P < 0.0001$ )

	$MT_{gel@ G'=0.5Pa}$	$MT_{gel@ G'=1Pa}$	$MT_{gel@ G'=5Pa}$	$MT_{gel@ G'=10Pa}$	$MT_{gel@ G'=15Pa}$	$F'_{tmin}$	$F''_{tmin}$	$F''_{tmax}$	$R'_{tmax}$	$R''_{tmax}$
$MT_{gel@ G'=1 Pa}$	0.999	-	-	-	-	-	-	-	-	-
$MT_{gel@ G'=5 Pa}$	0.996	0.997	-	-	-	-	-	-	-	-
$MT_{gel@ G'=10 Pa}$	0.987	0.99	0.997	-	-	-	-	-	-	-
$MT_{gel@ G'=15 Pa}$	0.952	0.956	0.972	0.986	-	-	-	-	-	-
$F'_{tmin}$	0.928	0.934	0.950	0.960	0.963	-	-	-	-	-
$F''_{tmin}$	0.909	0.917	0.937	0.951	0.962	0.992	-	-	-	-
$F''_{tmax}$	0.947	0.951	0.960	0.962	0.953	0.993	0.979	-	-	-
$R'_{tmax}$	0.906	0.910	0.927	0.940	0.955	0.987	0.978	0.979	-	-
$R''_{tmax}$	0.802	0.807	0.823	0.834	0.851	0.941	0.926	0.934	0.963	-
$R''_{tmin}$	0.911	0.912	0.916	0.910	0.892	0.937	0.921	0.957	0.932	0.875

**Industrial relevance:** The prototype fluorescence and infrared backscatter sensor investigated in this study combined with the developed rheological prediction model can be employed to monitor and control coagulation kinetics in a wide range of dairy processing applications including fresh cheese varieties and yoghurt manufacture.

**Highlights:**

- Feasibility of a novel ultraviolet excited fluorescence and infrared backscatter sensor to monitor acid induced coagulation kinetics of skim milk demonstrated
- Acid induced coagulation of skim milk was carried out at three different concentrations of glucono-delta-lactone (GDL)
- Measurement of coagulum storage modulus ( $G'$ ) was used as a reference rheological method to monitor coagulation kinetics
- Rheological models were developed using time parameters derived from the sensor response profiles to predict the times at which the acid induced skim milk coagulum reached selected  $G'$  values



Figure 1

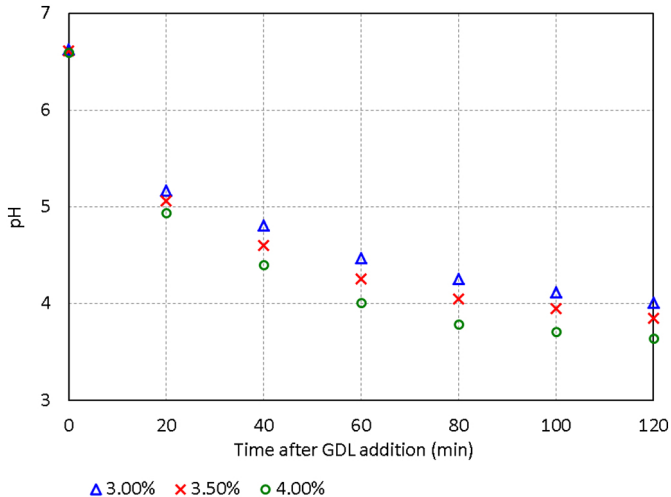


Figure 2



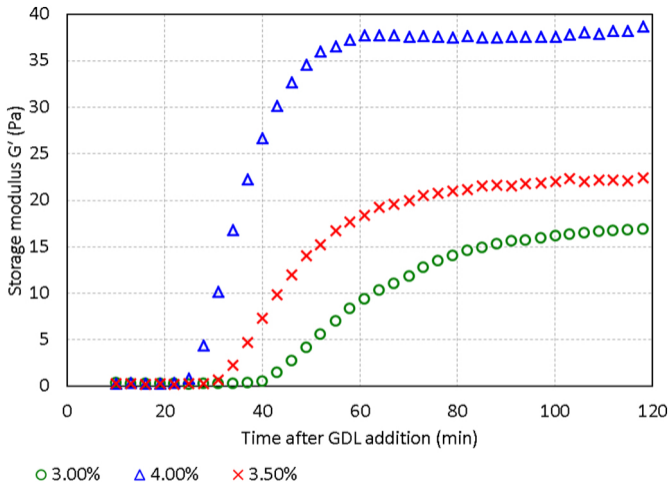


Figure 3

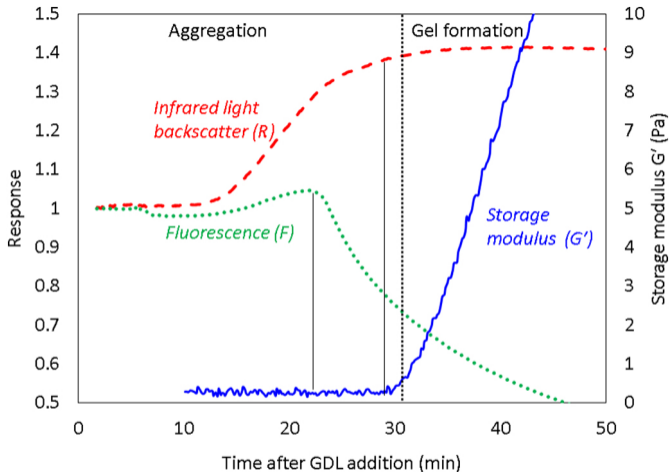


Figure 4

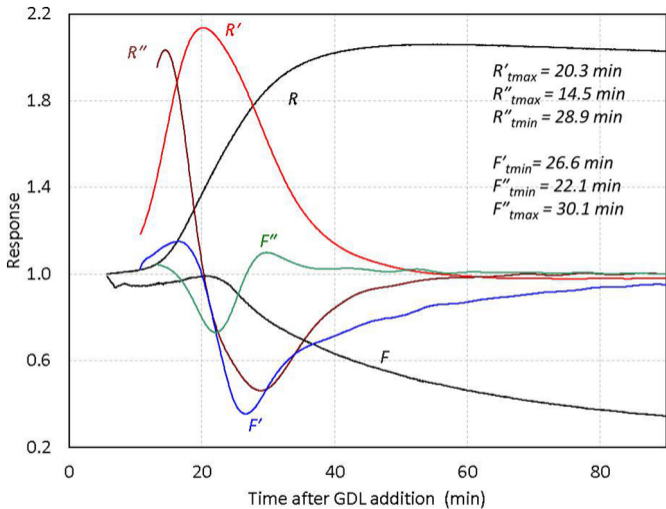


Figure 5

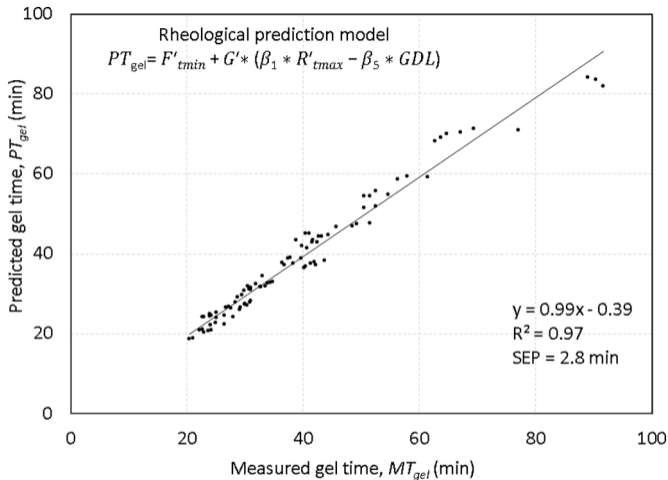


Figure 6