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International Dairy Journal 16 (2006) 147–152
INTERNATIONAL DAIRY JOURNAL
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Effect of temperature and inoculum concentration on prediction of both gelation time and cutting time. Cottage cheese-type gels

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Received 5 January 2004; accepted 23 February 2005

Abstract

Prediction of gelation and cutting times were investigated under conditions used in cottage cheese processing. Effect of temperature and inoculum concentration on predictions was analyzed using a factorial design. Culture growth was monitored using a pH-meter, an oscillatory rheometer and a light (880 nm) backscatter sensor. Gelation time determined by oscillatory rheometry was predicted by the equation $t_{gel} = \beta_1 t_{2\min}$ (determination coefficient, $R^2 = 0.988$ and standard error of prediction, SEP = 11.3 min), where β_1 was the regression coefficient and $t_{2\min}$ was the time to the first minimum of the second derivative of the light backscatter ratio. Cutting time was set as the time when gel pH reached 4.8 and predicted using the equation $t^*_{\text{cut}} = \beta_0 + \beta_1 t_{2\max 2}$ ($R^2 = 0.987$, SEP = 14.7 min), where β_0 and β_1 were regression coefficients and $t_{2\max 2}$ was the second maximum of the second derivative of the light backscatter ratio. The inclusion of an acidification term in the cutting time prediction model reduced the SEP to 7.9 min. These results demonstrated that, when the acidification rate varied, accurate cutting time prediction of cottage cheese required the inclusion of reference pH values.

Keywords: Cottage cheese; Light backscatter; Rheology; Gelation and cutting times

1. Introduction

In most fermented dairy products, such as yoghurt, sour cream and cottage cheese, the end point of the fermentation process is a decisive factor in the product quality (St-Gelais, Champagne, Erepmoc, & Audet, 1995). Cottage cheese constitutes an important segment of US cheese production representing ~8% of total cheese production (Dairy Products 2002 Summary, 2003). Several authors have reported that it is important to have a correct determination of the "optimum" cutting time to maximize cottage cheese texture, homogeneity and yield (Payne, Freels, Nokes, & Gates, 1998; Crofcheck, Payne, & Nokes, 1999). Firmness in this type of cheese primarily depends on pH, and to a lesser degree on rennet action, if rennet is used (Walstra, Geurts, Noomen, Jellema, & van Boekel, 2001). For this reason, during cottage cheese manufacture, the cutting time is usually determined by the cheese maker based on subjective evaluation of curd texture and, especially, on objective pH measurement. Cutting the curd at the right pH is the most important factor in producing a high-quality cottage cheese (Crofcheck et al., 1999). Acidification is generally allowed to proceed until a pH value of 4.6–4.8 is reached. If the gel is cut at pH values above 4.8 the curd grains are initially fragile but become overly firm and high in solids when cooked. When the curd is cut at pH values <4.6, its moisture content and fragility increases and there are excessive fines (Emmons & Beckett, 1984). When no rennet is used, the gel is ready to be cut at pH 4.6. When a small amount of rennet is

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doi:10.1016/j.idairyj.2005.02.006

used, it influences milk coagulation due to the hydrolysis of κ-casein with the result that gelation occurs sooner and at a higher pH than in the absence of rennet. Walstra et al. (2001) recommended cutting the curd at pH 4.8 when $2\,\mu L\,L^{-1}$ of rennet is used. A fibre optic probe was proposed by Payne et al. (1998) to monitor the changes in light (880 nm) backscatter during the fermentation stage of cottage cheese. These authors correlated two parameters generated by light backscatter (t_{max} or time to the maximum slope of the light backscatter change, and R'_{max} or slope at t_{max}) with the actual cutting time (t_{cut}) determined by a cheese maker in a manufacturing facility. The linear model tested was

$$t_{cut} = \beta_0 + \beta_1 t_{max} + \beta_2(R'_{max}), \quad (1)$$

where β_0, β_1, and β_2 were regression coefficients. The standard error of prediction (SEP) of the proposed model was 8.7 min. The SEP was not expressed in pH units, but it was evaluated by the authors as being too large to be used to automate the selection of curd cutting time. This error was attributed to the variability of activating the data acquisition system and the unknown variability in the operator's judgment of cutting time. An improved method was presented by Crofcheck et al. (1999) in which the SEP was reduced to 6.4 min using the prediction equation:

$$t_{cut} = t_{max} + \beta_2 S, \quad (2)$$

where β_2 was a regression coefficient and S was the difference in time between the maxima of the second and first derivatives of the light backscatter profile. In this model the previous parameter, R'_{max}, was substituted by the time parameter, S, which was less dependent on milk composition than the previous one. Payne et al. (1998) and Crofcheck et al. (1999) proposed the use of the light backscatter probe for early detection of acidification failure caused by bacteriophages, antibiotic residues, residual sanitizer compounds and natural bacterial inhibitors present in milk.

The effect of various factors (protein, calcium and enzyme concentration, pH, temperature and enzyme type) on cutting time prediction of enzymatic coagulation has been studied for cow milk (Payne, Hicks, & Shen, 1993; Ustunol, Hicks, Payne, & Milton, 1993) and goat milk (Castillo, 2001). Eqs. (1) and (2), which were proposed for cutting time prediction in cottage cheese, were developed from research in an industrial manufacturing facility where processing conditions were constant. The effect of various factors that influence coagulation and that cover the range of conditions used in cottage cheese making (i.e., short-, medium- and long-set methods) on cutting time prediction has not been investigated. In addition, alterations in the acidification rate may result from the normal variation of raw materials, pre-treatment of milk, biological nature of the coagulating system, human or technical mistakes, etc. All these factors affect coagulation and have a significant impact on cutting time. These variations create a need for an in-line sensor that can monitor coagulation and justifies an investigation of the effects of coagulation factors on the development of gelation time and cutting time prediction models. The development of an optical sensor technology that would be able to predict cutting time within a wide range of acidification rates would result in improved cottage cheese yield, quality and homogeneity. The goals of this study were to: (a) monitor the coagulation process of cottage cheese at the different acidification rates normally encountered in the industrial processing (short-, medium- and long-set) using a light (880 nm) backscatter sensor and a rheometer; (b) develop algorithms for predicting the gelation time determined from rheological tests and the curd cutting time (i.e., time when gel pH reaches 4.8).

2. Materials and methods

Data analyzed in this study correspond to the data set presented by Castillo, Lucey, and Payne (2005), where details of the materials and methods were explained. Only a brief description of aspects of special relevance is provided here. A randomized factorial experiment with two factors, i.e. starter concentration [0.5%, 2.75% and 5% (w/w)] and temperature (22, 27 and 32 °C), and three replications was performed at constant concentration of calcium chloride and chymosin. The coagulation process occurring at the different acidification rates was monitored simultaneously using a light backscatter sensor, a rheometer and a pH-meter until the pH value was 4.8. Light backscatter was monitored as described by Castillo et al. (2005). These authors also provided definitions of the light backscatter parameters used that were classified as suggested by Castillo, Payne, Hicks, and López (2000). Dynamic low amplitude oscillatory rheology and pH measurements were performed, and the corresponding parameters obtained, as described by Castillo et al. (2005). Different regression models for predicting the rheologically determined gelation time (t_{gel} or time when the gel had a storage modulus, $G' \geqslant 1$ Pa) and the cutting time (t^*_{cut} or time when the gel pH reached a value of 4.8), including independent variables and dependent variables generated from the light backscatter profile, were tested using the maximum R^2, GLM, and NLIN procedures of the Statistical Analysis System (SAS®, 1999).

3. Results and discussion

3.1. Prediction of the rheologically determined gelation time by light backscatter parameters

The maximum R^2 procedure of SAS was utilized to obtain the best one-, two- and three-parameter models for predicting the gelation time measured by the rheometer. Both independent variables (starter concentration, S_0, and temperature, T) and several of their functions ($1/S_0$, $\ln S_0$, T^2) were tested for the best R^2 together with dependent variables: time-based parameters ($t_{\max}$, $t_{2\max}$, $t_{2\min}$, $t_{2\max 2}$, $t_{2\min 2}$, and the difference between each two of these previous time parameters designated as t_1 to t_{10}); mixed-based parameters ($R'_{\max}, R''_{\max}, R''_{\min}, R''_{\max 2}, R''_{\min 2}$); response-based parameters ($R_{\max}$); the calculated parameter, $Rt = t_4/t_9$, where t_4 was $t_{2\min 2}-t_{\max}$ and t_9 was $t_{2\min}-t_{\max}$; and pH-based parameters (maximum slope of acidification, R_A, time to the maximum slope of acidification, t_{R_A} and pH values at t_{R_A}, $t_{\max}$, $t_{2\max}$, $t_{2\min}$, $t_{2\max 2}$, $t_{2\min 2}$). The best one-, two- and three-parameter models are shown in Table 1. Model I was the best one-parameter model having the time-based parameter $t_{2\min}$ and an intercept. Only the regression coefficient assigned to the term $t_{2\min}$ was significantly different from zero. The best two-parameter model was Model II and it included, in addition to $t_{2\min}$, the mixed-based light backscatter parameter $R''_{\max 2}$. The regression coefficient term for $R''_{\max 2}$ and the intercept were found to be insignificant, which indicates overfitting. Comparing Model I with Model II, the SEP was reduced by 0.6 min. Parameters affected by the sensor response, such as $R''_{\max 2}$, are considered to be less desirable for use in a prediction model because their magnitude is influenced by milk composition. The inclusion of $R''_{\min 2}$, a parameter that is also affected by the sensor response, in Model II yielded the best three-parameter model (Model III). Even though Model III had a smaller SEP, it was not considered practical for in-line prediction of gelation time due to the large number of significant coefficients needed to implement it and to the slight decrease in SEP that was observed, compared with Model I.

Castillo et al. (2000) found that the Berridge clotting time for goat milk was predicted by the equation $t_{\text{clot}} = -0.63 + 1.17 t_{\max}$. For that reason, a regression between $t_{\max}$ and t_{gel} was explored using the GLM procedure of SAS; the resulting Model IV had a non-significant intercept and a SEP that was 0.9 min larger than Model I, confirming that the parameter $t_{2\min}$ was a slightly better descriptor of t_{gel} than $t_{\max}$. Since the intercept was not significant in Model I or Model IV, they were simplified to Models V and VI. Model VI was preferred for an in-line application, because it was based on an easily measurable time-parameter and only needed a single regression coefficient to predict t_{gel}. The SEP for Model VI was initially viewed as being too large for an accurate prediction, but the SEP of 11.3 min corresponded to just 0.082 pH units. This suggested that the apparently large error between predicted and observed gelation time was not as decisive in terms of pH values.

O'Callaghan, O'Donnell, and Payne (1999) studied six different techniques of monitoring coagulation ("CoAgulite sensor" based on light backscatter measured at 880 nm, "Gelograph" and "TxPro" sensors using NIR transmission, the thermal "hot wire" sensor and vibrational sensors "Viscolite" and "Sofraser") and compared these techniques with each other and with the rheologically determined t_{gel}. The existence of a correlation between the inflection point of the profiles determined with each of the sensors and the rheological parameters, allowed the authors to predict t_{gel} with a SEP between 45 and 70 s (depending on the sensor considered). The average rheologically determined t_{gel} and the SEP for its prediction using the CoAguLite sensor were reported to be 1575 and 50 s, respectively, which corresponded to a coefficient of variation (CV) of 3.17%. The CV observed in the present work, using Model VI, was 3.77%. It was estimated under changing experimental conditions (S_0 and T) while the previous authors only changed the rennet concentration. Since Castillo et al. (2000) demonstrated that the effect of enzyme concentration in the prediction of Berridge clotting time was trivial, the small difference in the CV values for t_{gel} predictions observed by O'Callaghan et al. (1999) and the present work was considered to be irrelevant, suggesting that t_{gel} could be accurately predicted by light backscatter.

Castillo et al. (2005) suggested that the time-based light backscatter parameter $t_{2\min 2}$ (the time to the second minimum of the second derivative of the light

Table 1
Models for the prediction of the rheologically defined gelation time (t_{gel}) using light backscatter[a]

Model		β_0	β_1	β_2	β_3	R^2	SEP (min)
I***	$t_{gel} = \beta_0 + \beta_1 t_{2\min}$	4.39^{ns}	1.09***	—	—	0.989	11.4
II***	$t_{gel} = \beta_0 + \beta_1 t_{2\min} + \beta_2 R''_{\max 2}$	−41.2^{ns}	1.17***	72 052^{ns}	—	0.990	10.8
III***	$t_{gel} = \beta_0 + \beta_1 t_{2\min} + \beta_2 R''_{\max 2} + \beta_3 R''_{\min 2}$	−60.7*	1.21***	76 866*	142 226*	0.992	10.1
IV***	$t_{gel} = \beta_0 + \beta_1 t_{\max}$	4.35^{ns}	1.13***	—	—	0.987	12.3
V***	$t_{gel} = \beta_1 t_{\max}$	—	1.14***	—	—	0.986	12.2
VI***	$t_{gel} = \beta_1 t_{2\min}$	—	1.11***	—	—	0.988	11.3

[a] $N = 27$. Some of the most important light backscatter predictors are briefly defined in the text, but for detailed definition of parameters see Castillo et al. (2005). β_0, β_1, β_2, β_3, regression coefficients. R^2, determination coefficient (corrected for the means). SEP, standard error of predictions; ^{ns}Not significant at 95%; *Significant at 99%; ***Significant at 99.99%.

backscatter ratio against time) was not significantly different from t_{gel} and could be used to estimate t_{gel}. Thus, including $t_{2\min 2}$ into a cutting time prediction model essentially incorporates information about the beginning of the gelation process. Unfortunately, accuracy of $t_{2\min 2}$ determination depends on the sharpness of the second derivative second minimum. For those processing conditions where $t_{2\min 2}$ is not clearly distinguishable (i.e., not sharp enough) or is even absent (i.e., enzymatic coagulation at high temperatures, ~40 °C, and protein concentrations, ~7%) Model VI could represent a potential objective estimate of $t_{2\min 2}$ ($\sim t_{gel}$), which could be useful in improving cutting time prediction.

3.2. Prediction of the curd cutting time by light backscatter parameters

Models were tested for the prediction of curd cutting time, which for this test was defined as the time when the pH reached 4.8. The independent variables tested (S_0 and T), and some of their more relevant functions ($1/S_0$, $\ln S_0$, T^2), together with the dependent variables, were tested for the best R^2. Dependent variables investigated were similar to those used for t_{gel} prediction. Cutting time estimation was also studied "indirectly" through the prediction of the gel assembly period established by the difference $t^*_{cut} - t_{max}$. Since, the best one-, two- and three-parameter models for predicting $t^*_{cut} - t_{max}$ always gave a higher SEP than the respective models for t^*_{cut} prediction and included basically the same parameters, only the analysis corresponding to those models predicting t^*_{cut} are discussed below. Model I (Table 2) was the best one-parameter model. It included a significant intercept and a significant time-based parameter ($t_{2\max 2}$). The SEP for the prediction was high (14.7 min). Inclusion of a significant "pH-based" parameter (pH at $t_{2\min 2}$) reduced the observed SEP from 14.7 to 11.0 min, suggesting that acidification rate played an important role in the prediction of cutting time (which was expected as the cutting time target was pH 4.8). This fact was confirmed with the best three-parameter model. It included two pH-based parameters (pH values at t_{max} and at $t_{2\min 2}$). The inclusion of two terms accounting for the pH profile considerably reduced the SEP from 11.0 to 7.9 min. This result was expected as including two pH terms allowed the model to account not only for pH value but also for the acidification rate. Furthermore, inclusion of four more statistically significant factors to Model III (R_t, t_9, R''_{max} and R_{max}) reduced the error considerably ($R^2 = 0.999$, SEP = 5.4 min). Since the observed reduction in the SEP value (2.5 min) required obtaining 8 regression coefficients instead of 4, it was not considered practical for in-plant operation. It should be noted that when two pH-based parameters were incorporated to the prediction model, $t_{2\min 2}$ was a better predictor of cutting time, in terms of R^2, than $t_{2\max 2}$. The parameter $t_{2\min 2}$ was reported to be not significantly different from t_{gel} by Castillo et al. (2005). It was hypothesized that the presence of $t_{2\min 2}$ and pH at $t_{2\min 2}$ in Model III could be related to the strong relationship existing between this time-based parameter and t_{gel}.

Previous models proposed for cutting time prediction in cottage cheese (Payne et al., 1998; Crofcheck et al., 1999) were developed at constant processing conditions (i.e., constant starter concentration, enzyme concentration and incubation temperature), and where the acidification rate was largely consistent. For that reason, these models were tested in order to investigate their ability to compensate for the changes in acidification rate induced by various cottage cheese making procedures (i.e., short-, medium- and long-set). Model IV, which corresponded to the model (Eq. (1)) proposed by Payne et al. (1998) had a SEP of 15.1 min. This error was much greater than the one previously reported (8.7 min) by Payne et al. (1998). In order to compare the SEP values, the corresponding CV were calculated. The CV observed by Payne et al. (1998) for the prediction of t_{cut} using Model IV (2.68%) was smaller than the CV observed in the present study (3.65%) for the same model. This suggests that the effectiveness of this model for cutting time prediction decreased when processing conditions were changed. Model V, which was the equivalent to the model (Eq. (2)) proposed by Crofcheck et al. (1999), had a SEP of 23.7 min. This was very high

Table 2
Models for the prediction of cutting time (i.e., time when gel pH reaches 4.8) using light backscatter[a]

	Model	β_0	β_1	β_2	β_3	R^2	SEP (min)
I***	$t^*_{cut} = \beta_0 + \beta_1 t_{2\max 2}$	59.0***	1.25***	—	—	0.987	14.7
II***	$t^*_{cut} = \beta_0 + \beta_1 t_{2\min 2\,pH} + \beta_2 t_{2\max 2}$	−507***	102***	1.24***	—	0.993	11.0
III***	$t^*_{cut} = \beta_0 + \beta_1 t_{2\min 2\,pH} + \beta_2 t_{\max pH} + \beta_3 t_{2\min 2}$	−452***	322***	−225***	1.24***	0.997	7.91
IV***	$t^*_{cut} = \beta_0 + \beta_1 t_{max} + \beta_2 R'_{max}$	20.9[ns]	1.42***	1428[ns]	—	0.986	15.1
V***	$t^*_{cut} = \beta_1 t_{max} + \beta_2 S$	—	1.49***	2.12[ns]	—	0.972	23.7

[a] $N = 27$. Some of the most important light backscatter predictors are briefly defined in the text, but for detailed definition of parameters see Castillo et al. (2005). β_0, β_1, β_2, β_3, regression coefficients. R^2, determination coefficient (corrected for the means). SEP, standard error of prediction; [ns] Not significant at 95%; ***Significant at 99.99%.

compared with the SEP of 6.32 min reported by Crofcheck et al. (1999) using this model to predict t_{cut} under constant processing conditions. The regression coefficient β_1 of Model V was found to be very significant. For that reason it was not possible to assume that its value was unity, which indicated that it was not appropriate to simplify Model V to a simpler equation $t^*_{cut} = t_{max} + \beta_2 S$, as proposed by Crofcheck et al. (1999), when the acidification rate was changing. These results confirm that models without pH terms were not able to accurately predict t^*_{cut} at different acidification rates. Fig. 1 graphically compares the predictions of t^*_{cut} obtained by Models III, IV and V.

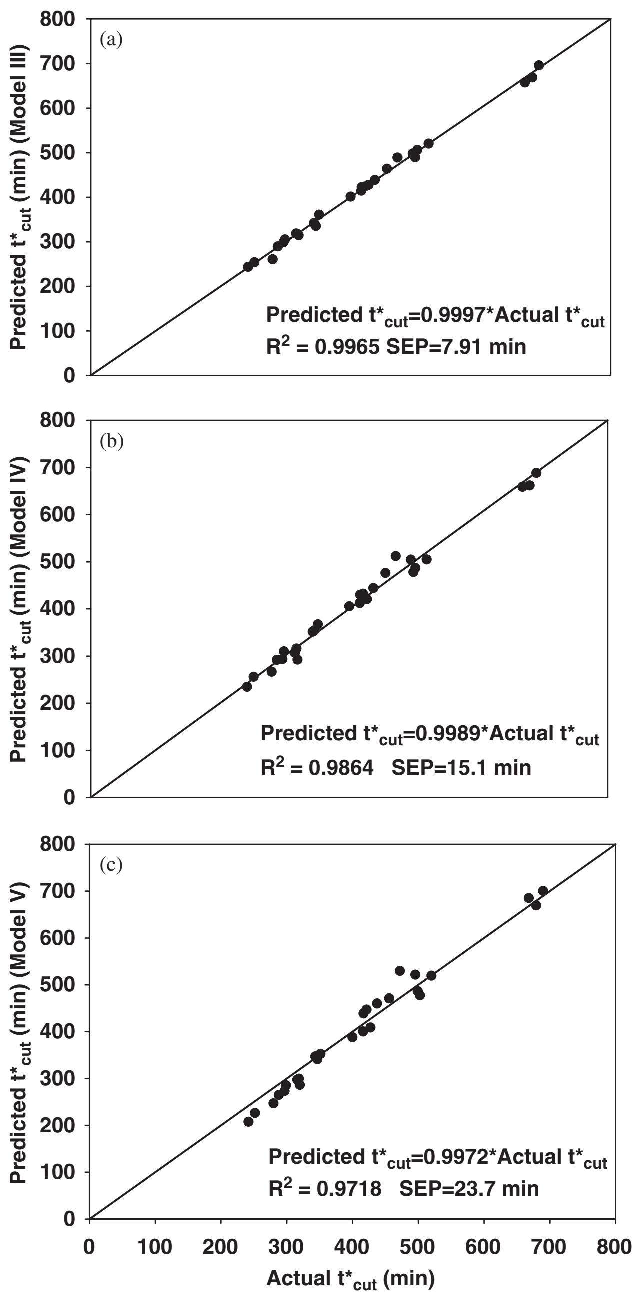

Fig. 1. Prediction of cutting time versus actual cutting time (cutting time defined as the time when gel pH reaches 4.8) using Models III (a), IV (b) and V (c). $N = 27$. R^2, determination coefficient (corrected for the means). SEP, standard error of prediction.

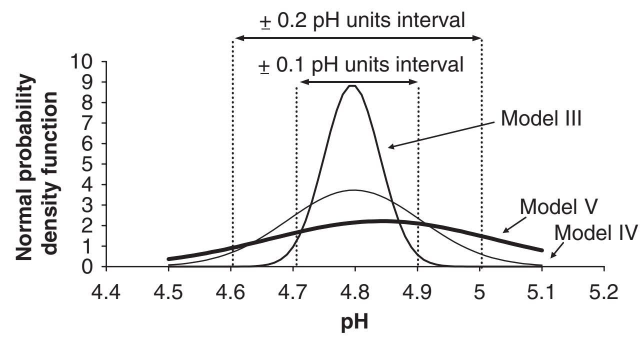

Fig. 2. Normal probability density function for the prediction of the pH 4.8 (cutting time) using Models III, IV and V.

There is another aspect that should be considered in order to effectively and practically evaluate the SEP for the different models tested. As was mentioned in the introduction, pH is considered to be the most important factor affecting the gel firmness in cottage cheese. For that reason, the evaluation of the models SEPs in pH units seemed to be important. For each test, the actual pH corresponding to the predicted cutting time yielded by Models III, IV and V, were obtained. The frequency distribution of the residuals (in pH units) for the three models tested was normal ($P > 0.05$). Fig. 2 shows the normal probability density function for the predictions of pH 4.8 obtained by the three models. The normal variables corresponding to the pH prediction obtained by each one of the three models were Model III prediction ~ Normal ($\mu = 4.79$, $\sigma = 0.045$), Model IV prediction ~ Normal ($\mu = 4.79$, $\sigma = 0.107$), Model V prediction ~ Normal ($\mu = 4.84$, $\sigma = 0.180$). The criterion selected for an acceptable cutting time prediction was that prediction values should yield residuals smaller than 0.1 pH units (Emmons & Beckett, 1984) in at least 95% of occasions. Using this criterion only Model III fulfilled this precision established for the prediction of cutting time. Since neither Model IV nor V included any term for pH, they could not accurately predict a specific pH value during the fermentation of cottage cheese when acidification conditions were changing. Thus, Model III was selected as the best model to predict the fermentation end-point in cottage cheese under changing processing conditions with a SEP of 0.045 pH units.

4. Conclusions

The rheologically determined gelation time was estimated using parameters derived from light (880 nm) backscatter with a SEP of 0.082 pH units. This confirmed that light backscatter at 880 nm was sensitive to both aggregation and gel firming steps. It was found that the rate of acidification affected the prediction of cutting time. The inclusion of two pH measurements at specific times ($t_{\max}$, $t_{2\min 2}$) into the cutting time prediction model was required to maintain the prediction error within ± 0.1 pH units under the conditions often encountered in cottage cheese processing with rennet addition.

Acknowledgements

The authors wish to thank the Wisconsin Center for Dairy Research (University of Wisconsin-Madison) for allowing 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 belonging to 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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