Preliminary Evaluation of an Optical Method for Modeling the Dilution of Fat Globules in Whey During Syneresis of Cheese Curd

M. Castillo, F. A. Payne, M. B. López, E. Ferrandini, J. Laencina

Abstract. *An optical sensor designed to measure whey fat concentration was tested on whey samples from a cheese processing plant. Whey samples were collected to determine syneresis kinetics at different times after gel cutting. Normalized spectral sidescatter intensity was measured by mean of a fiber optic spectrometer (300-1100 nm). A sidescatter waveband ratio ($S_{875/425}$) was calculated by dividing intensity at 875 by that at 425 nm. Whey fat concentrations were predicted by using the power low type equation previously developed by the above authors, [Fat] = β_0 ln (β_1 $S_{875/425}$ + β_2). Predicted whey fat concentrations were compared to actual concentrations measured by the Gerber method. The change in whey fat concentration with time after gel cutting was used to estimate the syneresis reaction rate. Results confirmed that fat dilution in whey followed a first order response. The light sidescatter technology for determining syneresis kinetics was considered to have potential but requires additional work to improve measurement accuracy.*

Keywords. *Process control, Inline sensor, Fiber optic, Fat concentration, Whey, Syneresis kinetics.*

Curd consists of a protein skeleton (casein network) and an interstitial fluid called whey. Syneresis is the expulsion of whey as the protein matrix contracts after the gel is cut. The contraction of the network results from bond rearrangement between casein aggregates (Van Dijk, 1982). At the end of syneresis, rigid curd grains and a large amount of whey are obtained. Control of the syneresis is a key step for increasing the curd yield and improving cheese quality (Castillo, 2001). After curd drainage, curd transforms into cheese as a result of enzymatic degradation of chemical compounds occurring during the ripening process (McSweeney and Fox, 1993). The extent of syneresis during cheese making controls the moisture, mineral, and lactose content of the curd. All of these factors affect cheese ripening and, subsequently, its final sensory and safety attributes. Many cheese defects result from high curd moisture content that decreases cheese grade, price, and shelf life. Some countries have regulations specifying maximum moisture content for each type of cheese. Very little information about the prevalence of cheese defects is available. A recent study (Smukowski et al., 2003) surveyed the U.S. cheese industry to determine the prevalence and economic impact of cheese defects. The percent of downgraded cheeses in 2001 was 7.26% and 16.80% for cheddar and Swiss cheeses, respectively, with estimated annual losses of $29 and $24 million. Improving curd moisture content control could eliminate part of this loss. Unfortunately, the status of syneresis is currently not measured during cheese production. Syneresis is empirically controlled, all over the world, by processing for specific temperatures and times, resulting in variation in the curd moisture content.

Successful development of a process control technology for syneresis control will require three developments. The first is a sensor technology to measure low fat concentrations in whey. Optical methods proposed for measuring low fat concentration in milk have been briefly reviewed by Castillo et al. (2005). They proposed a sensor technology to measure low whey fat concentration using the ratio of light sidescatter intensities at 875 to that at 425 nm ($S_{875/425}$) and the equation:

$$[Fat] = \beta_0 \ln (\beta_1 S_{875/425} + \beta_2) \qquad (1)$$

where $\beta_0 = -0.80$, $\beta_1 = -0.70$, and $\beta_2 = 1.66$. The second development is a technique for determining syneresis kinetics from whey fat dilution inline. Few authors have investigated the relation between whey fat concentration and whey separation during curd shrinkage (Castillo et al., 2000). The third required development is a model relating the extent of syneresis to curd moisture content.

The goals of this work were to: a) validate the optical method proposed by Castillo et al. (2005) for measuring low concentration of whey fat and; b) characterize the kinetic of whey fat dilution during syneresis.

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Materials and Methods

Sample Preparation

Goat cheese whey samples were collected during syneresis from Algodonera Cheese Plant (Murcia, Spain) on each of three testing days. A mesophilic lactic culture (*Streptococcus thermophilus*, *Lactococcus lactis* spp. *lactis* and *L. lactis* spp. *cremoris*; CHN22, Chr. Hansen S.A., Madrid, Spain) and anhydrous $CaCl_2$ (0.156 mg L^{-1}), both in water solution, were added to pasteurized (72°C to 75°C, 15 s) goat milk with continuous stirring. After 20 min (pH ∼6.4), milk was coagulated at 30°C by adding 0.3-mL kg^{-1} calf rennet (80% chymosin; 145 IMCU mL^{-1}) supplied by Caglio Star España, S.A. (Murcia, Spain). Once the gel was cut, the mix of curd and whey was cooked with continuous stirring by gradually increasing the temperature from 30°C to 38°C. Twelve 100-mL whey samples were collected at different times (∼2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 30, 45 min) after gel cutting. The whey samples were cooled, transported to the lab, and immediately filtered through a nylon cheesecloth (1-mm^2 pores) to remove the casein fines from the whey, warmed to ∼25°C, stirred and split in two aliquots. One aliquot was used to measure (in duplicate) fat concentration by Gerber method (IDF, 152A, 1997; accuracy of 0.05%) (GM) while a second aliquot was used to measure the normalized sidescatter spectral scan.

Optical Measurement of Fat Concentration

Whey fat concentration was estimated by the optical method proposed by Castillo et al. (2005). The authors reported that whey fat concentrations could be predicted with an R^2 of 0.99 and a CV of 6.27% by using the equation 1 within the range 0.0 to 0.9% fat. A fiber optic spectrometer (model SD2000, Ocean Optics, Inc., Dunedin, Fla.) and a prototype cell, designed to measure light sidescatter (90°), were connected by optical fibers and probes as shown in figure 1. Characteristics of the fiber optic spectrometer were described in detail by Castillo et al. (2005). The sampling cell consisted of a 40-mL cylindrical black plastic base with 2.54-cm diameter holes bored at 90° for inserting of the fiber optic probes. A black plastic cap avoided external light interferences. The distance between the fiber optic probes was set to 6 mm using a calibration rod spacer. The light source used was a tungsten halogen light source (LS-1, Ocean Optics, Inc.). The light source and the slave unit were connected by optical fiber probes and cables manufactured by the University of Kentucky and Reflectronics Inc. (Lexington, Ky.) using 400- and 600-μm diameter fibers (Spectran Specialty Optics, Avon, Conn.). The integration time was set (5 to 15 s) by the computer software (OOIBase, Version 1.5, Ocean Optics, Inc.) according to the light intensity. The spectral scan, S(λ), was automatically processed by subtracting the dark spectral scan and dividing by the integration time to give the normalized sidescatter spectral scan, SN(λ) (bits s^{-1}). Each SN(λ) was divided into 50-nm wavebands with mid−wavelengths in the range 375 to 1025 and the response averaged. Ratio $S_{875/425}$ was calculated by dividing the normalized sidescatter intensities at wavebands 875 and 425 nm.

Measurement of Whey Fat Dilution Kinetics

Whey samples collected at different times after gel cutting were analyzed for whey fat concentration. The first order syneresis kinetic rate constant was estimated by non-linear fitting (SAS®, 1999) of the measured whey fat concentrations as a function of time to the next equation:

$$[Fat] = [Fat]_{\infty} + ([Fat]_0 - [Fat]_{\infty})e^{-kt} \qquad (2)$$

where k was the syneresis kinetic rate constant (min^{-1}), t was time after cutting the gel (min), $[Fat]_0$ was fat concentration at time t = 0, and $[Fat]_{\infty}$ was the asymptotic whey fat concentration. Syneresis kinetic parameters estimated by fitting equation 2 to measured fat concentration were used as a reference. Equation 2 was also fitted to the optically predicted whey fat concentration data. This would validate the proposed optical whey fat measurement method for estimating fat dilution kinetic parameters.

Results and Discussion

Kinetics of Fat Dilution in Whey During Syneresis

The measured whey fat concentrations as a function of time after gel cutting were found to follow a first order

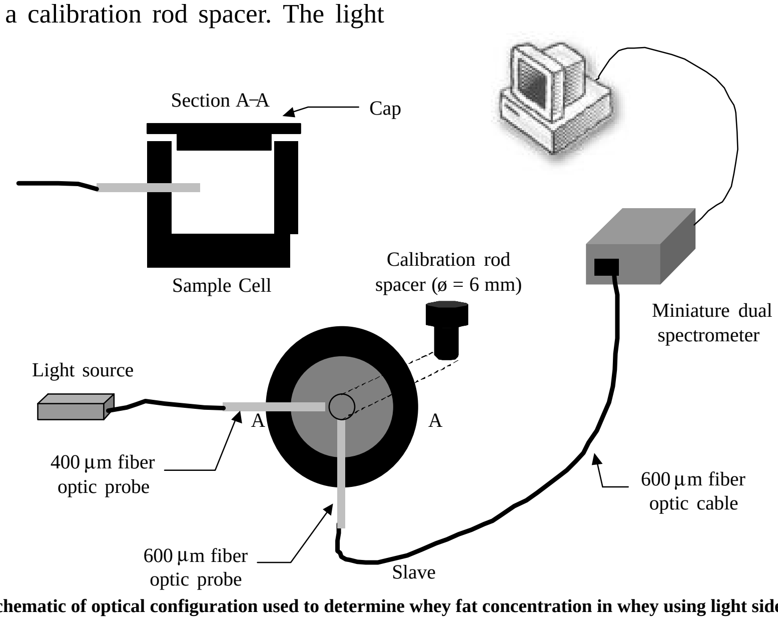

Figure 1. Schematic of optical configuration used to determine whey fat concentration in whey using light sidescatter at 90°.

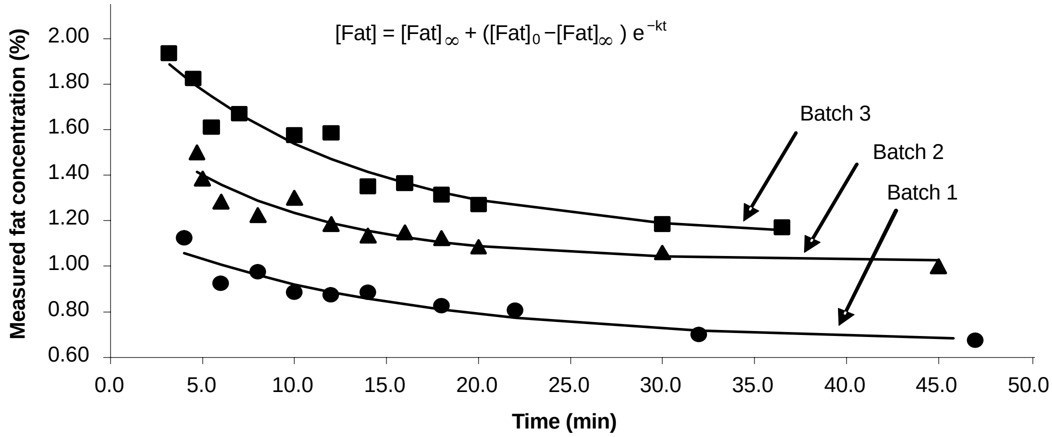

Figure 2. Plot of measured whey fat concentration as a function of time. Solid lines represent least squares fit of a first order kinetic equation (eq. 2) to the measured whey fat concentration. Data obtained during three different manufacturing processes of "Ripened Murcia Cheese." Whey fat concentration determined by Gerber method.

response, as shown in figure 2. Fat globules are trapped by the gel structure during syneresis and cannot diffuse through the casein network. In fact, most of the fat globules were liberated from curd into the whey at the moment of cutting and/or during the first minutes of stirring. Then, the exponential decay of whey fat concentration with time was as expected considering that: a) the expulsion of whey from the curd during syneresis is responsible for whey fat dilution; and b) whey flow from curd follows a first order kinetic reaction (Marshall, 1982; Peri et al., 1985; Noel et al., 1989; Castillo et al., 2000). The syneresis kinetic rate constants obtained by fitting equation 2 to actual whey fat concentrations are shown in table 1. The syneresis kinetic rate constants were in the range 0.09 ± 0.02 min^{-1}, in agreement with values observed by Castillo et al. (2000). Estimated initial whey fat concentration, $[Fat]_0$, and fat concentration at infinite time, $[Fat]_\infty$, were in the range of 1.68 ± 0.48% and 0.93 ± 0.24%, respectively. Thus, the whey fat concentration decreased ∼44% during syneresis. The R^2 values were in the range 0.91 ± 0.03. The differences between the syneresis kinetic constant between batches were attributed to differences on initial milk composition between batches and, especially, to small differences between the processing conditions between days.

Table 1. Kinetic parameters for the dilution of fat in whey during syneresis of goat milk cheese.[a]

Batch No.	Method	$[Fat]_0$ (%)	$[Fat]_\infty$ (%)	k (min^{-1})	R^2
1	Gerber	1.19	0.66	0.072	0.90
	OE[b]	1.13	0.60	0.035	0.85
	OER[c]	1.03	0.57	0.032	0.86
2	Gerber	1.70	1.02	0.116	0.89
	OE	1.75	0.97	0.050	0.82
	OER	1.45	0.85	0.036	0.84
3	Gerber	2.14	1.12	0.089	0.94
	OE	2.54	0.94	0.048	0.89
	OER	2.15	1.09	0.082	0.86

[a] Parameters obtained by non-linear fit of measured whey fat concentration (Gerber method) or optically estimated whey fat concentration to a first order kinetic equation (eq. 2). Whey fat estimated using eq. 1 as proposed by Castillo et al. (2005).

[b] OE, optical estimation of whey fat.

[c] OER, optical estimation of whey fat after re-calibration (see text for further details).

Optical Prediction of Whey Fat Concentration

Whey fat concentration data were used to validate the optical fat measurement method proposed by Castillo et al. (2005). Equation 1 was used to predict the whey fat concentration of each sample using the sidescatter waveband ratio, $S_{875/425}$. The measured and predicted whey fat concentrations are shown in figure 3. It shows that batch one (●) fits better than batches two and three (▲). The prediction had an R^2 of 0.89 and a CV of 7.68% (batch one). Note that most of the data points corresponding to batches two and three were out of the calibration range for equation 1 (0 to 0.9%) developed by Castillo et al. (2005) and yielded non-accurate fat concentration predictions. For that reason, equation 1 was re-calibrated to the measured whey fat concentration data and resulted in an R^2 of 0.95 and a CV of 6.17% (fig. 4). The calibration parameters obtained (β_0 = -0.90; β_1 = -0.63; β_2 = 1.60) were reasonably similar to those determined by Castillo et al. (2005). These results suggest that light sidescatter ratio $S_{875/425}$ can be used to estimate whey fat concentration in the range 0 to 2%. However, the predictive equation may need to be calibrated to obtain appropriate regression coefficients within the actual whey fat concentration range.

Optical Prediction of Whey Fat Dilution Kinetic Parameters During Syneresis

The predicted whey fat concentration values estimated by the fiber optic method proposed by Castillo et al. (2005) (eq. 1) were also fitted to equation 2 to estimate the kinetic parameters for whey fat dilution. Table 1 summarizes the estimated kinetic parameters and compares them to the reference kinetic parameters determined for whey fat measurement (GM). It is observed that the results for the syneresis kinetic rate constant, k, was clearly underestimated. The inaccuracy for k prediction results from both scatter in actual fat concentration data points probably associated with whey sampling (fig. 2) and the relatively small decrease in fat concentrations during cooking (∼44%). These results suggest that accurate estimation of the syneresis kinetic rate constant would require a more reliable sampling method, and improvement of whey fat concentration prediction accuracy. Increasing the number of samples collected at each specific time and averaging their corresponding fat concentration values could contribute to

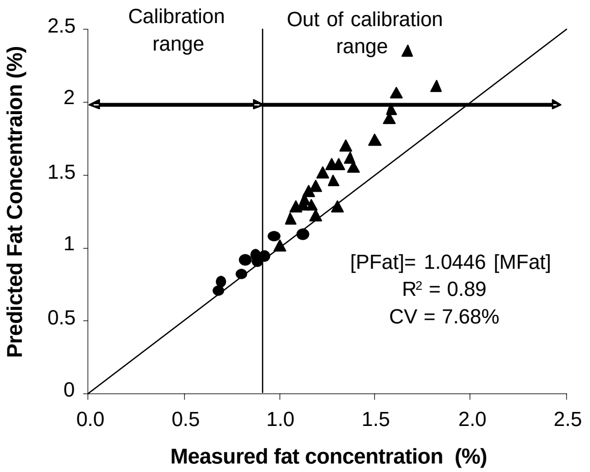

Figure 3. Prediction of whey fat concentration using the sidescatter ratio $S_{875/425}$ and equation 1. [PFat] is predicted fat; [MFat] is measured fat (Gerber method); N = 34; and R^2 and CV values corresponded exclusively to the prediction of fat concentration for batch number one (●). Data points for batches two and three (▲).

reducing the inaccuracy introduced by the sampling method. Increasing the data points as a function of time may also contribute to improve the accuracy of *k* prediction. Improvement of whey fat concentration prediction accuracy would require changes in the optical configuration of the light sidescatter sensor. However, the main objective for the proposed optical technology is not to directly estimate the kinetic parameters for fat dilution during syneresis but rather to obtain fat dilution kinetic parameters related to kinetics of curd shrinkage that allow estimating the curd moisture content during cheese processing. Thus, the accuracy of kinetic rate constant estimation is not as decisive as long as proportionality between estimated fat dilution kinetic parameters and curd shrinkage exists. Further research at different syneresis rates needs to be addressed to establish the existence of such proportionality.

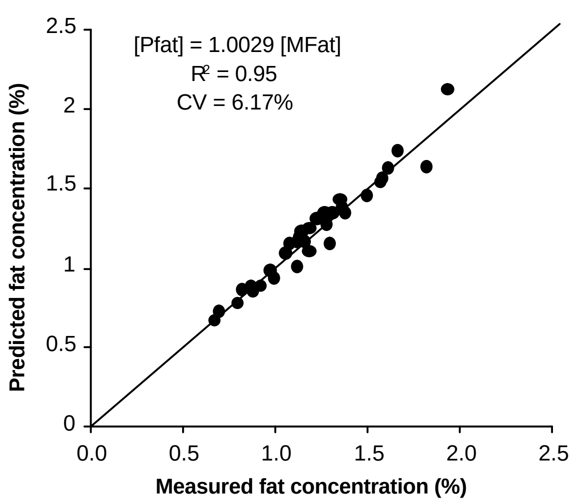

Figure 4. Fit of whey fat concentration and sidescatter ratio $S_{875/425}$ to equation 1. [PFat] is predicted fat; [MFat] is measured fat; N = 34; and R^2 and CV values corresponded to the fit of the three experimental batches.

Conclusions

Results of this preliminary study show that dilution of fat globules in whey after cutting the gel followed a first order reaction. This study also showed that the sidescatter ratio, $S_{875/425}$, is sensitive to whey fat concentration in the 0 to 2% range allowing the prediction of whey fat concentration with an R^2 of 0.95 and a CV of 6.17%. These findings suggested that the development of an inline sensor technology for measuring whey fat, based on sidescatter ratio, $S_{875/425}$, is feasible but needs further research.

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