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Diffuse Reflectance Changes During the Culture of Cottage Cheese

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DIFFUSE REFLECTANCE CHANGES DURING THE CULTURE OF COTTAGE CHEESE

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ABSTRACT. *A sensor for measuring diffuse reflectance of milk during the typical 6-h culture of cottage cheese was installed in a local manufacturing facility. Diffuse reflectance was found to increase slowly during the first three hours of the culture and increase rapidly toward the end of fermentation. The correlation between parameters generated from the diffuse reflectance profile and cutting time was sufficient to develop an algorithm for cutting time prediction. An algorithm incorporating t_{max} (time from adding culture to the maximum rate of change in reflectance) and slope of the reflectance curve at t_{max} predicted the operator selected cutting time with a standard error of 8.7 min.* ***Keywords.*** *Coagulation, Sensor, Fiber optic, Milk, Cheese.*

Cottage cheese is formed by acid coagulation of skim milk using either lactic acid fermentation or direct acidification. Coagulation of the milk proteins can result exclusively from lactic acid fermentation, or, more practically, by lactic acid fermentation supplemented by enzymatic coagulation. With either method, once the curd is formed it is cut, cooked in its own whey, drained, washed, blended with a salted dressing, and packaged.

Cutting the curd at the right time is a critical operation in cottage cheese manufacture. Cheese plant operators select cutting time using pH and a subjective observation of curd texture. Incorrect selection of cutting time has ruined many batches of coagulum. It is desirable to cut the curd at a pH near the isoelectric point of casein where casein is most insoluble and easily precipitated. Theoretically, this point is 4.7 for casein (Eck, 1986). The pH at cutting determines the physical characteristics of the curd (Emmons and Beckett, 1984; Perry and Carroad, 1980). Curd cut at pH values above 4.8 is initially fragile, but when cooked is overly firm and high in total solids. Curd cut at pH values below 4.6 cooks out too soft, disintegrates into excessive fines, and yields a high moisture content curd (Emmons and Beckett, 1984). Although pH is the single most important factor in measuring culture progress, it is difficult to measure consistently in a production facility because of the inherent variability within and between pH meters. Detection of starter culture failures during the early stage of the culture when corrective action can be taken is difficult when only pH is used to measure the progress of curd formation. Failures of the bacterial starters occur frequently and are caused by factors such as bacteriophage, antibiotic residues, residual sanitizing or cleaning compounds and natural inhibitors present in milk (Varnum and Sutherland, 1994).

Calcium is essential in the formation of curd when the casein is precipitated by the combined action of rennet and lactic acid at the isoelectric point of casein. Calcium is generally added to skim milk because calcium may be precipitated during pasteurization or may be low when the cows are on spring grass.

Changes in light reflectance have been studied for monitoring enzymatic coagulation. A fiber optic probe that monitors the change in diffuse reflectance of infrared radiation at a wavelength of 950 ± 5 nm was reported by Ustunol et al. (1991) for use in monitoring enzymatic coagulation of milk. The use of fiber optics to monitor changes during milk coagulation has received commercial interest (Damrow Company, Fond du Lac, Wis., and Reflectronics Inc., Lexington, Ky.). The small size and flexibility of optical fibers have opened new opportunities for making measurements where conventional apparatus do not exist or cannot be used because of the sanitation, size, or electronic constraints. The reflectance ratio profiles of coagulating milk had a definitive shape depending on the chemical and physical state of the coagulum. The reflectance ratio profile, as shown in figure 1, was divided into three periods: (1) induction, (2) sigmoidal, and (3) logarithmic (Payne et al., 1993). Little change occurred during the induction period. Casein precipitation and agglomeration began during the sigmoidal period. The point at which flocculation was visible occurred near the end of the sigmoidal period or beginning of the logarithmic period. The first derivative of the reflectance profile reached a maximum at t_{max}, the inflection point of the sigmoidal period (Payne et al., 1993). The t_{max} was found strongly correlated to the cutting time t_c for hard cheese production. The cutting time prediction model used for enzymatic coagulation was $t_c = \beta \times t_{max}$ where β was a

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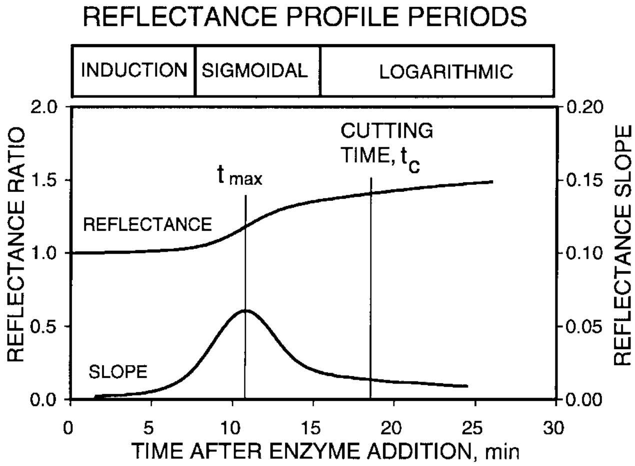

Figure 1–Typical reflectance ratio profile for enzymatic coagulation of milk.

constant selected to match the cheese plant operator's selection of cutting time (Payne, 1995).

Diffuse reflectance of milk was found to be significantly affected by pH (Lochte, 1995). This effect was considered to result from the change in shape of casein micelles with pH. Micellar casein dissociation and micelle voluminosity depend on pH (Tanford, 1968; Van Hooydonk et al., 1986; Wong et al., 1988). Visser et al. (1986) defined five stages of milk acidification between pH 6.5 and 4.5 at 43°C. Between pH levels of 6.6 and 5.5, casein micelles are found with a wide range of diameters. At a pH of 5.5, sufficient calcium and phosphate groups have been released such that the original and smaller casein aggregates become visible. At a pH of 5.2, an aggregation of corpuscle structure is observed. As the pH is lowered further, these aggregates seem to contract into smaller areas and finally individual casein particles are formed again, larger than the original casein micelle and different in character resulting from the loss of calcium phosphate.

Considering the fact that diffuse reflectance of milk is affected by both pH and enzymatic hydrolysis of casein one can see that cottage cheese processing which involves both a pH change induced by bacterial growth and enzymatic hydrolysis could be a more difficult process to predict than enzymatic hydrolysis alone. However, unlike hard cheese production, the culture of cottage cheese has a sufficient duration (typically 6 h as compared to 30 min for hard cheese) that a monitoring system may provide an early warning of problems and provide an opportunity to make corrective changes.

Objectives

The goal of this research was to improve consistency and efficiency of cottage cheese manufacturing through the development of technology for monitoring culture growth.

The specific research objectives were to:

1. Characterize changes in diffuse reflectance of cottage cheese during coagulation induced by culture growth.
2. Determine if an algorithm based on parameters generated from the diffuse reflectance curve can predict cutting time of the coagulum.

Materials and Methods

A fiber optic probe, designed to measure diffuse reflectance of milk, consisted of two optical fibers with one fiber emitting light and the other fiber returning reflected light to a photo detector as shown in figure 2. This optical sensor configuration measures light backscatter from the particulates in suspension (casein and fat particulates in this case) and is thus sensitive to changes in the backscatter characteristics of the casein particles. The probe was installed in a commercial cottage cheese making plant (Winchester Farms Dairy, Winchester, Ky.) on 26 March 1996. The optics, electronics and fiber optic probe were designed and fabricated by the Biosystems and Agricultural Engineering Department at the University of Kentucky. Figure 3 shows the main components of the data collection system. The optical fibers in the probe were connected to the sensor enclosure. The sensor enclosure was installed above the vat and contained the emitter and detector. The probe was removable from the vat for cleaning. A 10 cm × 10 cm baffle was attached to the 6.4-mm diameter stainless steel probe. A baffle was added to the probe to prevent vibration resulting from the movement of the mechanical support during the culture process. Experience had shown that vibration of the probe could prematurely separate the

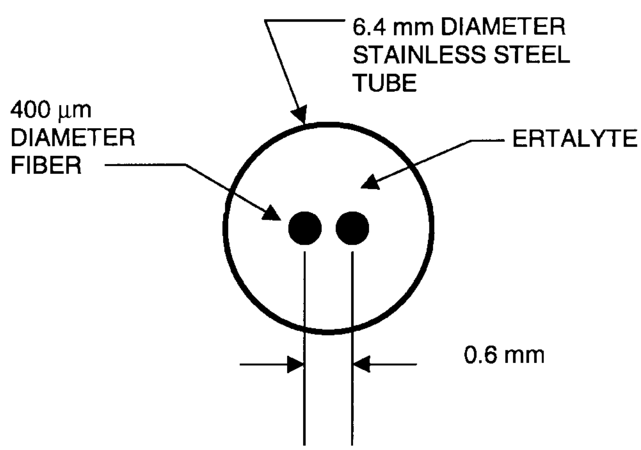

Figure 2–Schematic of fiber optic probe tip. The optical fiber located in the center carries light from the emitter. The optical fiber located 0.6 mm from the center transmitted reflected light to a photodetector.

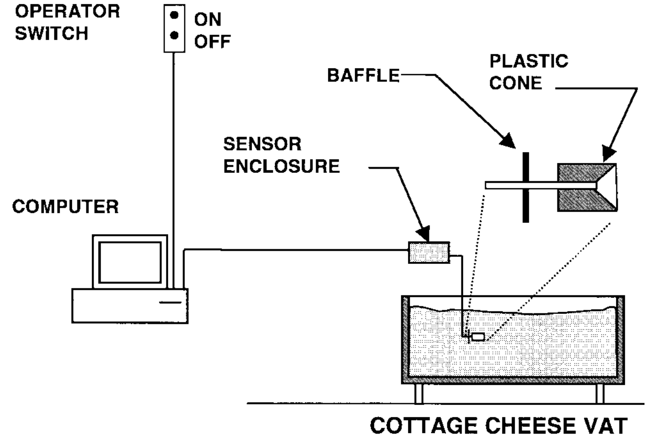

Figure 3–Schematic of data collection system for monitoring culture of cottage cheese.

whey from the curd locally in front of the probe and thus distort the reflectance signal after flocculation. A plastic cone, as shown in figure 3, was installed at the probe tip to further prevent movement of the curd around the distal tip during the process. The probe was placed into the vat before filling with milk. The cheese plant operator activated the data acquisition system after milk covered the probe tip. The operator used pH measurement and a subjective evaluation of the curd texture to determine cutting time. The operator deactivated the data acquisition system at cutting time.

The data acquisition system for monitoring cottage cheese culture consisted of a personal computer (486, 33 MHz) equipped with a Keithley-Metabyte CTM-05/A counter timer board and programmed for data acquisition using Visual Basic 4.0 (Microsoft Corporation) and VTX 1.1 (Keithley-Metabyte).

Data for each culture were collected and automatically stored by the computer. Data collected included process start time, process end time, and the reflectance measurement from the fiber optic probe. Reflectance was measured with an optical sensor (TSL235, Texas Instruments, Austin, Tex.) mounted to the fiber. The TSL235 output was a digital pulse (50% duty cycle) with the pulse frequency proportional to light irradiance. Pulse frequency (Hz) was collected every 6 s during the culture process and the average of 10 measurements (one minute of data) recorded. Reflectance ratios were calculated by dividing measured reflectance by the initial reflectance (averaged over the first 10 min after adding culture).

Winchester Farms Dairy also provided skim milk processing data sheets and cottage cheese setting information sheets. The information used from these data sheets included casein content, temperature of the milk during culture, initial pH in the vat, amount of calcium chloride added, and enzyme add time.

Skim milk was analyzed by Winchester Farms Dairy for casein content and pH. The processing temperature was held at 32.8°C. The skim milk was typically fortified with nonfat dry milk (NFDM) to raise total solids content to approximately 10%. Skim milk (11356 kg) was pumped into a vat after pasteurization and the vat temperature held at 32.8°C (91°F). Frozen culture (3.5 kg, Mesophilic Homofermentative O-Culture, R604 or R603 from DVS, Chr. Hansen's Laboratory, Inc. 9015 West Maple Street, Milwaukee, WI 53214) was added to the vat during filling. The dairy plant alternated daily between Culture Type 603 and 604 to avoid buildup of phage. Calcium chloride (624 g) was added to the skim milk at the beginning of culture. The calcium chloride and culture were mixed in the skim milk by continuous vat agitation during the first part of culture (approximately 3 h). Once the pH of the skim reached 6.0, 85 g of rennet was added. Vat agitation was continued 15 min after rennet addition. The pH was checked hourly and the curd was cut once the pH decreased to approximately 4.7. The curd was allowed to heal for approximately 30 min after cutting. The curd was then cooked in its own whey, drained, washed, and blended with a dressing.

Results and Discussion

A total of 27 cottage cheese vats for the production of lowfat or nonfat cottage cheese were monitored between

Table 1. Summary of data collected at Winchester Farm Dairy

Test No.	Date	Culture Type	Process Temp (C)	Calcium Chloride (g)	Initial pH	Casein (%)	t_{max} (min)	Enzyme Add Time (min)	RR* at t_{max}	Slope at t_{max} (min^{-1})	RR* at Cut Time	Observed Cut Time (min)
1	4-15-96	603	32.8	624	6.43	2.833	267	NA	1.352	0.0173	1.860	326
2	4-16-96	604	32.8	624	6.46	2.398	252	NA	1.649	0.0193	1.860	311
3	4-17-96	603	32.8	624	6.53	NA	273	NA	1.368	0.0185	1.855	325
4	4-18-96	604	32.8	624	6.32	2.548	267	NA	1.330	0.0175	1.720	338
5	4-21-96†	604	32.8	624	6.46	NA	296	NA	1.392	0.0186	1.758	357
6	4-22-96†	603	32.8	624	6.50	2.876	263	NA	1.373	0.0176	1.910	326
7	4-24-96†	603	32.8	624	6.52	NA	296	NA	1.368	0.0188	1.812	339
8	4-25-96	604	32.2	624	6.51	2.799	272	NA	1.289	0.0177	1.765	326
9	4-28-96	603	32.8	624	6.43	NA	292	NA	1.247	0.0149	1.692	349
10	4-29-96†	604	32.8	624	6.58	NA	304	NA	1.385	0.0193	1.823	359
11	5-2-96†	604	32.8	0	6.29	NA	282	NA	1.377	0.0195	1.823	319
12	5-15-96	603	32.8	624	6.42	2.840	299	NA	1.497	0.0201	1.991	342
13	5-16-96	604	32.8	624	6.38	NA	264	NA	1.513	0.0215	2.026	316
14	5-19-96	603	32.8	624	6.46	NA	283	NA	1.506	0.0196	1.995	324
15	5-20-96	604	32.8	624	6.51	3.090	268	NA	1.443	0.0224	1.891	309
16	5-22-96	604	32.8	624	6.52	2.780	313	NA	1.425	0.0193	1.837	348
17	5-23-96	603	32.8	624	6.35	2.870	285	NA	1.439	0.0195	1.890	324
18	6-2-96	603	32.8	624	6.48	3.010	222	181	1.566	0.0216	2.070	268
19	6-5-96	604	32.8	624	6.56	3.160	307	205	1.600	0.0235	2.098	346
20	6-6-96	604	32.8	624	6.60	3.120	257	191	1.627	0.0238	2.099	297
21	6-9-96	603	32.8	624	6.55	2.850	284	177	1.639	0.0248	2.134	311
22	6-10-96	604	32.8	624	6.48	NA	256	243	1.609	0.0283	2.137	283
23	6-12-96	604	32.2	624	6.50	2.899	257	NA	1.607	0.0274	2.151	297
24	6-20-96	604	32.8	624	6.48	NA	295	171	1.499	0.0215	1.985	339
25	6-23-96	603	32.8	624	6.46	NA	274	163	1.374	0.0221	1.787	317
26	6-26-96	603	32.8	624	6.50	NA	280	161	1.425	0.0202	1.838	334
27	6-27-96	604	32.8	624	6.50	NA	276	182	1.489	0.0217	1.993	346
Average			32.758		6.47	2.862	277	186	1.459	0.0206	1.919	325
Std Dev			0.203		0.07	0.199	19.7	23.9	0.111	0.0030	0.134	21.5

* RR = Reflectance Ratio.
† Estimated process start time—data not available.

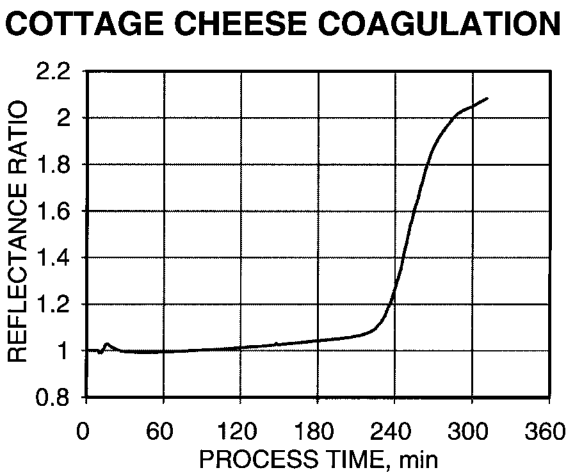

Figure 4–Typical reflectance ratio profile for cottage cheese production.

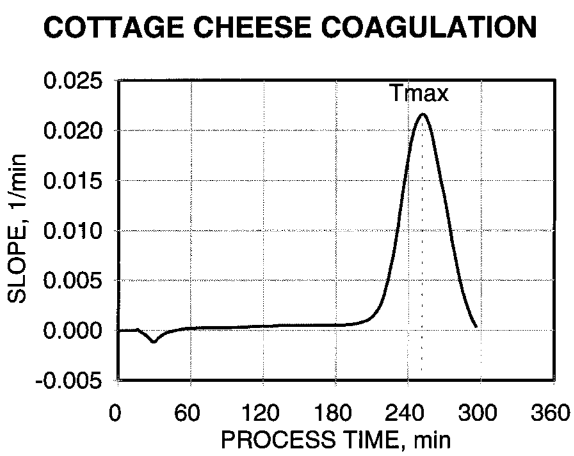

Figure 5–Slope of the reflectance ratio profile showing a peak at t_{max}.

26 March 1996 and 27 June 1996. The reflectance and production data are summarized in table 1. Casein was measured on 14 of the runs. The exact start time was estimated on five tests because the data acquisition system was started after the culture was added to the vat. The reflectance ratio (RR) showed variability during the early stages of the culture period that was thought to result from agitation and/or bubbles near the probe. However, the RR responses consistently showed a t_{max}. Table 1 shows t_{max}, cutting time (t_c), reflectance ratio at t_{max}, slope at t_{max}, reflectance ratio at cutting, and the process data from the plant data sheets for each test.

The reflectance profiles for 16 April 1996 are shown in figures 4 and 5. The reflectance ratio as shown in figure 4 slowly increased with time and then increased at a much faster rate beginning approximately 235 min into the culture process. This large increase continued for approximately 30 min then began to diminish. The curd was cut at a process time of 311 min thus ending the reflectance monitoring. The reflectance ratio nearly doubled during the culture process. The average reflectance ratio for the 27 tests (table 1) at cutting was 1.919, i.e., a 92% increase. This compares with an increase of 30 to 50% for enzymatic coagulation of milk for hard cheese production (Payne et al., 1993).

The slope (first derivative) of the reflectance ratio is shown in figure 5. The slope increased slowly until approximately 210 min into the culture process when it increased more rapidly, peaked, and then decreased. The peak slope occurred at 252 min (t_{max}) into the culture process. The initial milk pH averaged 6.47 with a standard deviation of 0.07 pH units. The casein content averaged 2.862% with a standard deviation of 0.20%.

The three parameters determined from the reflectance profile were t_{max}, reflectance ratio (RR) at t_{max}, and slope at t_{max}. The measured values of t_{max} had an average of 277 min and a standard deviation of 19.7 min. The observed cutting time had an average of 325 min and a standard deviation of 21.5 min.

The simplest model for predicting the cutting time is a linear model with zero intercept. Thus, the prediction model using only t_{max} would be:

$$t_{pred} = \beta \times t_{max}$$

Linear regression of this model on the collected data using the GLM procedure of SAS (1995) gave the following results:

$\beta = 1.172$	$s(\beta) = 0.009$	$P = 0.0001$
SEP = 12.07 min	N = 27	$R^2 = 0.72$

where
- β = Least squares regression coefficient
- $s(\beta)$ = standard error of estimate for β
- P = probability that the F-test statistic will exceed its observed value
- SEP = standard error of prediction
- R^2 = sum of squares corrected for the means
- N = number of data used in the regression

Several different linear models were tested with the independent variables cottage cheese type, culture type, initial pH, calcium chloride addition, casein content, enzyme add time and process temperature, in combination with t_{max} for predicting cutting time. The model having the least standard error of prediction from the independent variables tested contained casein content in combination with t_{max} and appeared as follows:

$$t_{pred} = \beta_o + \beta_1 \times \text{Casein} + \beta_2 \times t_{max}$$

The regression results for this model were:

$\beta_o = 186.8$ min	$s(\beta_o) = 48.9$	$P = 0.0029$
$\beta_1 = -31.5$	$s(\beta_1) = 13.7$	$P = 0.0415$
$\beta_2 = 0.81$	$s(\beta_2) = 0.12$	$P = 0.0001$
SEP = 10.17 min	N = 13	$R^2 = 0.82$

Linear models were tested on all parameters generated from the reflectance profile. The model which had the lowest standard error of prediction contained t_{max} and the slope at t_{max} and appeared as follows:

$$t_{pred} = \beta_o + \beta_1 \times t_{max} + \beta_2 \times \text{slope at } t_{max}$$

The regression results for this model were:

β_o = 154.0 min	$s(\beta_o)$ = 30.2	P = 0.0001
β_1 = 0.818	$s(\beta_1)$ = 0.09	P = 0.0001
β_2 = –2708.7	$s(\beta_2)$ = 584	P = 0.0001
SEP = 8.7 min	N = 27	R^2 = 0.85

This model has the advantage that only parameters generated from the reflectance profile are used to predict the cutting time. Thus, there is no need for input of milk composition data such as casein content. The parameter t_{max} requires knowledge of the culture starting time and determination of the peak slope. The culture addition is a manual operation and will require operator input. The peak slope can be determined by a number of algorithms. There are disadvantages to the use of slope at t_{max} in the above algorithm. Slope is dependent on the sensitivity of the fiber optic probe. The closer the fibers the stronger the signal. Using slope at t_{max} in a predictive algorithm would impose repeatability requirement on the design of a fiber optic probe. In addition, a change in fat content or solids content changes the reflectance characteristic of milk and would thus affect the slope at t_{max}.

The standard error of 8.7 min is viewed as relatively large and is likely too large to be used to automate the cutting time selection. However, even with this limitation the above algorithm could be used to alert the operator that a vat is nearing its cutting time. Thus, the algorithm may have value for plants (such as the Winchester Farms plant) that have seven vats for one operator to monitor.

Potential factors that may have contributed to the standard error include the variability of activating the data acquisition system and selecting the cutting time. The accuracy of culture start time was determined by the operators' timeliness of activating the system. In addition, the above statistical analysis inherently assumes that the operator's judgment of cutting time is correct. In reality, his subjective judgment has an unknown variability. Variability in the composition and pH of the milk may have also affected the microbial growth dynamics of the culture. Although initial pH and casein content were measured, there was insufficient variability to detect any significant correlation.

Future research should be directed toward development of a measure of slope that is independent of milk composition, reduction of the reliance on the operators' manual input for recording culture addition and cutting times. In addition, reflectance ratio profiles during the first three hours of culture should be investigated to determine if deviations from normal indicate culture problems.

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CONCLUSIONS

Diffuse reflectance changes during cottage cheese culture were measured for 27 tests at a local manufacturing facility. The reflectance ratio curves gradually increased during the first three hours of the culture. This was followed by a rapid increase in the reflectance ratio at around 4 h into the culture process. The time of maximum rate of change in reflectance (t_{max}) occurred typically at 277 min after starting the culture. Diffuse reflectance ratio increased by an average of 92% during the culture process.

Several linear models for predicting the cutting time were tested. A model containing t_{max} and slope at t_{max} predicted the operator selected cutting time with a standard error of 8.7 min. Although the standard error of prediction of 8.7 min is considered too large for complete automation of the cutting time selection, it does provide a method for alerting the cheese maker of a pending task requiring attention.

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