

AN ABSTRACT OF THE THESIS OF

Rachel A. Miller for the degree of Honors Baccalaureate of Science in Food Science and Technology presented on January 30, 2012. Title: Light backscatter: Shedding new light on milk coagulation.

Abstract Approved: _____
Lisbeth Goddik

Milk collected from individual cows (n= 31) was centrifuged to equalize fat composition and was tested during chymosin-induced coagulation to compare the influences of somatic cell level, breed, parity, protein composition, and lactation stage on coagulation rate. Coagulation rate was determined using diffuse reflectance. Pearson correlation coefficients were calculated for all quantitative variables, of which only lactation stage was statistically significant. A student's t-test was performed to compare coagulation rates between milk with somatic cell levels above and below 200,000 cells per mL. There was no evidence to suggest a difference in coagulation rates between the two somatic cell levels. Coagulation of Jersey milk was significantly faster than Holstein milk. Results from multivariate linear regression identified protein composition and breed as significant predictors of coagulation rate. Initial pH of the whole milk was also significant even though the pH was adjusted to 6.55 for all milk samples. The influence of milk age was also considered, using individual cow milk samples held for zero to five days. Coagulation time did not vary significantly over the five day period. Overall, the results of this study indicate that breed, lactation stage, initial pH, and protein composition significantly impact milk coagulation rate.

Key words: Milk coagulation, diffuse reflectance, Somatic Cell Count, Breed, Parity

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Light Backscatter: Shedding new light on milk coagulation

by Rachel A. Miller

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

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TABLE OF CONTENTS

	Page
INTRODUCTION.....	1
MATERIALS AND METHODS.....	9
Milk collection.....	9
Sample preparation.....	9
Milk composition analysis.....	10
Enzyme preparation.....	11
Coagulation time measurement.....	11
Effect of milk age on coagulation rate.....	12
Controlling error.....	12
Statistical analysis.....	13
RESULTS.....	14
Initial pH of milk samples.....	16
T _{max} analysis.....	17
T _{2min} analysis.....	19
Effect of milk age on coagulation time.....	20
Comparison of LactiCheck and Foss compositional analysis.....	21
DISCUSSION.....	23
CONCLUSION.....	27
REFERENCES.....	27

LIST OF FIGURES

Figure	Page
1. Mechanistic rationale behind the CoAguLab sensor.....	5
2. Relationship between light backscatter ratio and T_{\max}	7
3. Relationship between somatic cell count and pH of whole milk sample ($P < 0.001$).....	16
4. Comparison of T_{\max} values for milk samples from (n=6) individual cows over a five day period.....	20
5. Comparison of fat composition of whole milk as determined by by the Foss and LactiCheck methods.....	21
6. Comparison of the protein composition of (n=11) whole milk samples as determined by the Foss and LactiCheck compositional analysis methods.....	22

LIST OF TABLES

Table	Page
1. Distribution of DIM and parity among Holstein (n=18) and Jersey (n=13) cows.....	14
2. Summary of individual cow (n=31) age, breed, somatic cell count, days in milk, parity, initial pH, and skim milk composition as determined by LactiCheck ultrasonic milk analyzer.....	15
3. Calculated Pearson correlation coefficients and their corresponding p-values assessing the relationship of log somatic cell count, days in milk, initial pH, and skimmed milk fat and protein composition to T_{\max} (n=31) and $T_{2\min}$ (n=26).....	17
4. Linear regression analysis with breed, skim milk protein, and initial pH as predictors of T_{\max}	18
5. Results of student's t-test comparing T_{\max} values obtained for Holstein and Jersey milk, low somatic cell count Holstein and Jersey milk, low and high somatic cell count for both Holstein and Jersey, and differences in pH between low and high somatic cell count milk.....	19

Light Backscatter- Shedding new light on milk coagulation.

INTRODUCTION

Milk coagulation ability and rate represent critical components in the cheese making process. As a result, gel formation has been widely studied. Typically, milk coagulation is induced by addition of chymosin. Enzymatic coagulation is well studied and understood. The process occurs in two general steps: enzymatic hydrolysis of κ -casein (CN) and aggregation of rennet-altered micelles (Lucey, 2002). Overall, lower pH, increasing temperature, and sufficient concentrations of calcium encourage milk coagulation (Dalglish, 1983; Castillo *et al.*, 2006).

Milk somatic cell count (SCC) level has many documented effects on milk quality with regards to coagulation ability, presence of off-flavors, cheese yield, degree of lipolysis and proteolysis, and curd firmness (Barbano *et al.*, 1991; Klei *et al.*, 1998; Andreatta *et al.*, 2007). In general, increases in SCC are correlated with a reduction in cheese yield and curd firmness and an increase in coagulation time (Politis *et al.*, 1988; Ali *et al.*, 1980). This is likely due to the reduction of intact proteins in high SCC milk available for curd formation. However there is some debate as to whether or not SCC levels impact milk coagulation time. Several studies have shown an absence in correlations between SCC and milk coagulation time (Bastian *et al.*, 1991; Wedholm *et al.*, 2006; De Marchi *et al.*, 2007).

One of the main implications of elevated SCC levels in milk is its relationship with mastitis. Subclinical mastitis (**SCM**) is often diagnosed with increases in SCC (Hagnestam-Nielsen *et al.*, 2009). Mastitis remains a recurring problem for milk producers and processors alike. The onset of infection is accompanied by the migration of polymorphonuclear neutrophils (**PMN**) into the mammary gland (Harmon, 1994). During a mastitis infection, the production of proteases, reactive oxygen species, and cytokines that mediate inflammatory immune responses, increases in milk (Mehrzhad *et al.*, 2005). Other changes in milk composition include increases in immunoglobulins, serum albumin, lactoferrin, sodium, and chloride levels (Harmon, 1994). Several studies have indicated a correlation between pH and SCC (L. Okigbo *et al.*, 1985a; Politis *et al.*, 1988; Albenzio *et al.*, 2004). Harmon (1994) notes that the dramatic increase in the movement of blood components into the mammary tissue during mastitis is the primary reason for the increase in pH.

The various influences of breed, parity, lactation stage, season, and heritable milk coagulation properties (**MCP**) have been well studied by traditional methods (Bastian *et al.*, 1991; De Marchi *et al.*, 2007; Cassandro *et al.*, 2008; Wedholm *et al.*, 2006). Bastian *et al.* (1991) found that breed, season, and lactation stage significantly impacted rennet clotting time (**RCT**) as determined by use of a formagraph. The study found that Jersey milk clotted faster than Holstein milk, and also had a higher curd firmness. This is in agreement with Okigbo *et al.* (1985a) who showed that both abnormal and normal Jersey milk coagulated faster than normal Holstein milk. De Marchi *et al.* (2007) also found a significant difference in coagulation ability of milks from different breeds. The effect of parity on MCP is unclear. Ikonen *et al.* (2004) determined that milk from primiparous

cows had a lower curd firmness than milk from multiparous cows of the same breed while Bastian *et al.* (1991) did not identify a significant difference in curd firmness among cows with different parities after adjusting for protein and fat. Because milk composition changes throughout lactation, lactation stage, or days in milk (**DIM**) is thought to significantly influence MCP (De Marchi *et al.*, 2007; Bastian *et al.*, 1991; Tyriseva *et al.*, 2004). Tyriseva *et al.* (2004) determined that milk coagulation time was slowest at mid-lactation. Bastain *et al.* (1991) determined that clotting time decreased in late lactation as a result of increases in protein and fat. On the other hand, Okigbo *et al.* (1985b) showed that milk coagulation time was fastest in early lactation, and increased as lactation progressed. De Marchi *et al.* (2007) found that coagulation time increased as lactation stage increased. Therefore, although it is thought that lactation stage significantly influences coagulation time and curd firmness, the exact relationship remains unclear. With respect to seasonal variations in RCT, Bastian *et al.* (1991) found that RCT was significantly prolonged in the winter months, while De Marchi *et al.* (2007) found that RCT was fastest in September and October.

The impact of the age of raw milk has also been investigated with regards to RCT. In accordance with legislation, raw milk can be held for up to 48 hours on the farm and for up to 72 hours once it reaches the processing facility. It would therefore be advantageous for processors to know how the age of the raw milk influences coagulation rate. Forsback *et al.* (2011) found that milk collected from individual cows had the fastest mean coagulation time two days after collection. Leitner *et al.* (2008) found that RCT decreased as storage time increased.

Most of the influences of breed, parity, lactation stage, and season have been studied using traditional methods such as the formagraph or other rheometers (Okigbo *et al.*, 1985a; Wedholm *et al.*, 2006; Bastian *et al.*, 1991; De Marchi *et al.*, 2007; Lucey, 2002). However little research has been done to examine the applications of novel milk coagulation sensors to measure how these influences impact RCT. As the development of on-line sensors increases, their uses in industrial settings have as well. Several non-destructive sensors have been developed to monitor milk coagulation, with the advantage of leaving the curd intact (Lucey, 2002). One such on-line sensor is the CoAguLab optical coagulation measurement apparatus (Reflectronics; Lexington, Kentucky). The sensor uses changes in light backscatter (**LB**) of infrared light to monitor milk coagulation (Castillo *et al.*, 2003). As the enzymatic cleavage of the micelles proceeds, the diffuse reflectance, or LB ratio (**R**) increases while the micelle network forms (Payne & Castillo, 2007). Figure 1 shows a schematic representation of how the sensor monitors changes in diffuse reflectance so that inferences about milk coagulation can be made.

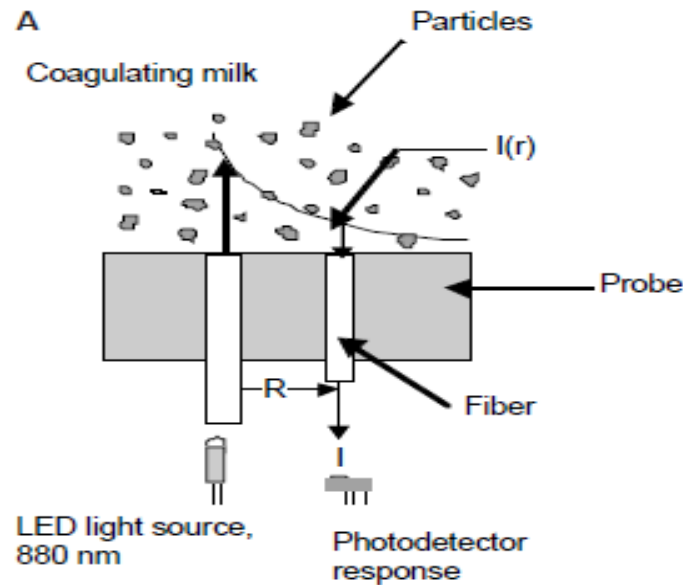


Figure 1: Mechanistic rationale behind the CoAguLab sensor- A schematic representation of the methods by which the diffuse reflectance profile is generated by the sensor to monitor milk coagulation (Image from Payne and Castillo, 2007).

Milk coagulation as measured by diffuse reflectance, possesses a characteristic sigmoidal shape (Payne & Castillo, 2007). The sigmoidal shape is the result of three general “stages” in the two-step reaction scheme. At first, R remains constant as the enzyme commences cleavage of the κ -caseins (Payne & Castillo, 2007). As enzyme hydrolysis proceeds, cleaved micelles begin to aggregate, and R increases as the growing micelle network reflects greater amounts of light (Payne & Castillo, 2007). Finally, the newly formed network rearranges itself to stabilize hydrophobic interactions and reduce electrostatic repulsion (Lucey, 2002), during which the LB ratio is still increasing, but at a less rapid and more constant rate (Payne & Castillo, 2007). Light backscatter primarily provides information about the rate of milk coagulation through the parameter T_{\max} ,

which is defined as the inflection point of the sigmoidal curve produced as the milk coagulation proceeds (Payne & Castillo, 2007). In other words, T_{\max} defines the time after rennet addition, at which rennet-altered casein particles are aggregating at their most rapid rate. Therefore, T_{\max} directly measures coagulation rate, and allows for inferences of coagulation time to made. Another parameter $T_{2\min}$ marks the start of the gel firming process, as determined by significant correlations with traditional rheological methods (Castillo *et al.*, 2006). Therefore, inferences about the rates of enzymatic hydrolysis and subsequent casein aggregation can be made (Payne & Castillo, 2007).

The CoAguLab records changes in R at 0.1 minute intervals. Because the first derivative requires a certain quantity of data points in order to constitute a line function from which the derivative can be established, the program uses the backscatter ratios from the initial two minutes to equate R' , and the time required to reach T_{\max} is thus recessed by two minutes and must be adjusted accordingly.

Due to its precise measuring capabilities of milk coagulation and the information yielded from the various stages of coagulation, diffuse reflectance was selected as the method used in this study to examine variations in milk coagulation time and rates.

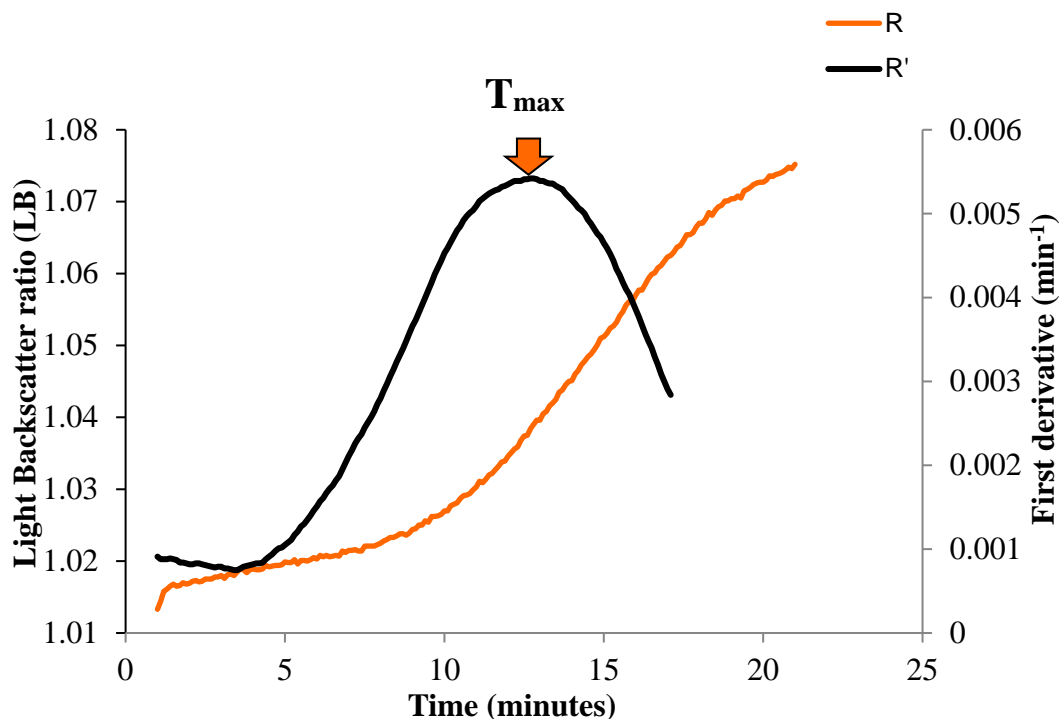


Figure 2: Relationship between light backscatter ratio and T_{\max} - Changes in the light backscatter ratio are plotted against time. The first derivative is used to calculate the parameter T_{\max} which represents the rate at which the light backscatter ratio is increasing at its most rapid rate.

Although the influences of DIM, breed, SCC level, and parity are well documented, the impact of these properties remain contradictory as determined by traditional methods. Therefore, there is a documented need for alternative methods to provide insight into how these properties impact milk coagulation rate. In addition, most of the studies examining MCP from individual cows did not equalize pH or fat content, which both have documented effects on milk coagulation rate. Thus, pH and fat represent confounding variables that could have potentially disguised the true impacts of DIM, breed, parity, and SCC level on RCT. The goals of this study were therefore to utilize a novel technique to

determine how DIM, breed, parity, and SCC level impacted the coagulation rate of milk collected from individual cows with adjusted pH and fat levels, and to also discern how the holding time of raw milk influences coagulation rate.

MATERIALS AND METHODS

Milk Collection

Morning whole milk samples were collected (approximately 500 mL) from 13 Jersey and 18 Holstein cows in the Oregon State University Dairy Research herd from October 2011 through November 2011. Cows were selected randomly for testing to include a range of DIM, age, parity, and SCC levels. Samples were collected using the Afimilk automated milking system (AfiLab, S.A.E Afikim, Israel) and were stored in 600 mL plastic containers prior to use. Milk samples were transported on ice in an insulated cooler to Oregon State University for testing. One gallon of pasteurized skim milk was purchased from a local store each week and was used as a control sample for each analysis.

Sample Preparation

Whole milk samples were inverted 20 times prior to use. Inverted milk samples were poured into two separate plastic containers to achieve a weight of approximately 240 grams each. Whole milk aliquots were also reserved for compositional analysis. Due to the variability in fat contents, samples were centrifuged to equalize fat contents. Samples were centrifuged in a Sorvall Super Speed Automatic refrigerated centrifuge (Model RC2-B; Wilmington, DE) at 8°C for five minutes at 3000 rpms without use of a brake. Centrifuged samples from identical individual cows were pooled into clean 600 mL plastic containers (approximately 350 grams) using cheese cloth to filter fat, and were inverted 20 times to mix. The pH of each milk sample was measured using an Accumet

Dual Channel pH/Ion meter (Fisher Scientific; Hanover Park, IL) model sensor, and starting pH values were recorded for each cow and store bought milk sample tested. The electrode was calibrated everyday prior to use using 4.00 and 7.00 buffer solutions stored at room temperature. Solutions of 1 M HCl (EMD Chemicals; Philadelphia, PA) and 1M NaOH (Sigma Aldrich; St. Louis, MO) were used to adjust pH of the milk samples until the desired pH of 6.55 was reached. A pH level of 6.55 was selected to reflect the typical pH of cheddar cheese processing. Samples were then stored at 2°C until use. The pH of the control sample was adjusted to pH 6.55 as well.

Milk Composition Analysis

Samples were also submitted to a certified laboratory testing facility for fat, protein, and SCC analysis using a MilkoScan Combi Foss (Foss; Hillerod, Denmark). Approximately 50 mL of milk (both pre and post-centrifugation) were stored in two ounce plastic snap-cap vials, and were preserved with one drop of 2-bromo-2-nitropropane-1,3-diol (D&F Control Systems, Inc.; Dublin, CA). Preserved milk samples were stored at 2°C for one to two weeks, and were shipped overnight on ice in insulated shipping containers.

Approximately 20 mL of inverted milk was collected from each individual cow milk sample before and after centrifugation and prior to pH adjustments. Samples were placed into a 32°C water bath until the temperature reached the required temperature of 15-25°C. Warmed samples were inverted several additional times, and were analyzed by LactiCheck ultrasonic milk analyzer according to the recommended procedure (Page & Pedersen International, Ltd.).

Enzyme Preparation

Enzyme-induced coagulation was done using recombinant chymosin donated by Chr. Hansen's Laboratory Inc. (Milkwaukee, WI). Approximately 0.300 g of chymosin was added to a 50 mL glass volumetric flask. A sodium acetate buffer (pH 5.5) prepared from sodium acetate trihydrate granules (Mallinckrodt Chemicals; Philipsburg, NJ) was added to the volumetric flask, to bring the total volume to 50 mL, giving a final enzyme concentration of 0.006 grams of enzyme per mL of milk. All enzyme solutions were prepared using chymosin from the same batch, with an activity of 637 IMCU/mL. The enzyme solution was made each testing day and was stored at 2°C prior to use to retain enzyme strength.

Coagulation Time Measurement

Coagulation time and diffuse reflectance profile of each milk sample were measured using the Model 1 CoAguLab optical coagulation measurement apparatus (Reflectronics, CoAguLab Model 1; Lexington, Kentucky). Aliquots of 80.0 g of chilled, centrifuged milk were added to each of the two CoAguLab measurement vessels. The added milk was allowed to equilibrate to 32.0 ± 0.3 °C, and was stirred for 10 seconds prior to addition of enzyme. Exactly 1.000 g of enzyme solution was added to each milk sample, and was stirred for 15 seconds to ensure even distribution of the enzyme. Data collection was started immediately after the enzyme addition. Individual cow samples were run simultaneously in duplicate, and the coagulation times were averaged. The temperature of the vat was kept constant at 32.0 ± 0.3 °C using a refrigerated circulator system (VWR

International, Model 1162A; Radnor, PA), and milk samples were allowed to coagulate until approximately five minutes after T_{\max} was reached.

Effect of Milk Age on Coagulation Rate

On day zero, during the morning milking, approximately 2.4 L of milk was collected from two Jersey and four Holstein cows. Each of the individual milk samples was divided into six 600 mL plastic containers at the farm, and were transported on ice to the laboratory for analysis. Samples were stored at 2°C until use. One sample from each cow was analyzed daily for days zero through five. Fat standardization and pH adjustment of each milk sample was done the day of analysis.

Controlling Error

Originally, whole milk samples were tested for their MCP. However, the absence of a correlation between T_{\max} and SCC level suggested that other variables could have been disguising any relationships. The high variability in fat composition from individual cows was therefore equalized to account for fat's role in milk coagulation rate. Because enzyme concentration has a high influence on milk coagulation rate, enzyme concentrations were equalized by weighing exactly 1.000 g of enzyme. Milk samples were also weighed to obtain more precise amounts than could have been obtained from volumetric methods. The temperature of the milk was kept at a constant $32.0 \pm 0.3^\circ \text{C}$ to lessen the impact of fluctuations in temperature on the enzymatic rate.

Statistical Analysis

Statistical analysis was done using MiniTab 16 statistical software (Minitab Inc.; State College, PA) and MedCalc Software (MedCalc Software; Mariakerke, Belgium). A student's t-test was used to analyze differences in T_{\max} between SCC levels above and below 200,000 cells per mL, and between Jersey and Holstein. A multivariate stepwise linear regression analysis was performed to determine association between cow, breed (0-Holstein and 1-Jersey), DIM, lactation number (dummy variables for lactations 1,2 and 3+), SCC level, and initial pH on T_{\max} and $T_{2\min}$. An alpha level of 15% was used for entering and removing variables. A Passing-Bablok regression analysis was used to evaluate relationships between protein and fat measurements between the two analytical methods.

RESULTS

A total of 31 cows were included in the data analysis, including 18 Holstein and 13 Jersey cows. Distribution of parity was: 5 first lactation cows (all Holstein), 16 second lactation cows (n=9 Holstein, and n=7 Jersey), and 10 cows in their third lactation or higher (n=4 Holstein, and n=6 Jersey). Table 1 lists the average DIM by lactation and breed group. The average DIM for Holstein and Jersey was 152.39 and 88.15 days respectively. Table 2 lists the compositional data of the milk, age, parity, and DIM of the cows included in the study.

Table 1. Distribution of DIM and parity among Holstein (n=18) and Jersey (n=13) cows.

Lactation group	Holstein cows			Jersey cows		
	Avg	±	S.D.	Avg	±	S.D.
1	146.67	±	161.79	-		
2	160.88	±	47.63	95.00	±	91.22
3+	144.00	±	93.83	80.17	±	33.96
Total	152.39	±	101.23	88.15	±	68.56

Table 2. Summary of individual cow (n=31) age, breed, somatic cell count, days in milk, parity, initial pH , and skim milk composition as determined by LactiCheck ultrasonic milk analyzer.

Cow I.D.	Somatic Cell Count x 1000	Breed	Days In Milk	Age of Cow	Parity	pH Whole Milk	Skim Milk Fat	Skim Milk Protein
1	596	Holstein	212	3.08	2	6.95	0.38	3.55
2	123	Holstein	233	3.08	2	6.88	0.06	3.44
10	1,697	Holstein	114	3.05	2	6.82	0.15	3.41
17	53	Holstein	161	3.02	2	6.90	0.12	3.41
22	290	Holstein	126	3.01	2	6.80	0.31	3.58
27	7	Holstein	118	3.00	2	6.72	0.20	3.61
45	14	Holstein	89	2.04	1	6.76	0.26	3.63
48	84	Holstein	124	2.03	1	6.77	0.27	3.69
52	64	Holstein	107	2.01	1	6.86	0.08	3.60
54	55	Holstein	56	2.00	1	6.76	0.17	3.59
58	91	Holstein	34	2.00	1	6.70	0.04	3.59
239	79	Jersey	49	7.03	7	6.80	0.04	3.56
269	318	Jersey	70	6.06	4	6.93	0.05	3.71
275	74	Jersey	38	3.11	2	6.76	0.06	3.73
289	184	Jersey	45	5.06	4	6.91	0.11	3.68
290	708	Jersey	121	5.06	4	6.98	0.12	3.43
298	12	Jersey	74	5.03	4	6.88	0.21	3.45
329	36	Jersey	122	4.02	3	6.72	0.10	3.87
331	1,962	Jersey	285	4.02	2	7.13	0.17	3.80
339	1,259	Jersey	120	3.08	2	7.53	0.04	3.08
350	59	Jersey	34	3.04	2	6.83	0.14	3.81
354	180	Jersey	37	3.03	2	6.88	0.18	3.72
355	103	Jersey	108	3.02	2	6.83	0.06	3.66
359	63	Jersey	43	3.02	2	6.80	0.12	3.65
792	1,460	Holstein	208	9.02	8	6.87	0.09	3.51
905	69	Holstein	95	6.06	4	6.85	0.26	3.62
947	71	Holstein	236	5.05	3	6.86	0.19	3.41
949	2,200	Holstein	37	5.04	4	7.20	0.03	3.16
983	130	Holstein	199	4.02	2	6.69	0.19	3.72
990	476	Holstein	124	3.11	2	6.81	0.35	3.37
991	285	Holstein	470	4	1	6.80	0.21	3.77

Initial pH of milk samples

The pH of each milk sample was recorded prior to analyses. The mean initial pH was 6.87 ± 0.17 (with a range of 6.69 to 7.53). The pH of milk from $n=11$ cows with high SCC ($> 200,000$ cells per mL) was significantly higher ($P=0.028$) than the pH of milk from $n=20$ cows with low SCC ($<200,000$ cells per mL). Results of the ANOVA identified pH as a significant source of variance among different SCC levels ($P<0.001$). A somewhat linear relationship was observed between SCC and initial pH. Figure 3 displays the linear relationship between initial pH of whole milk samples and log SCC levels.

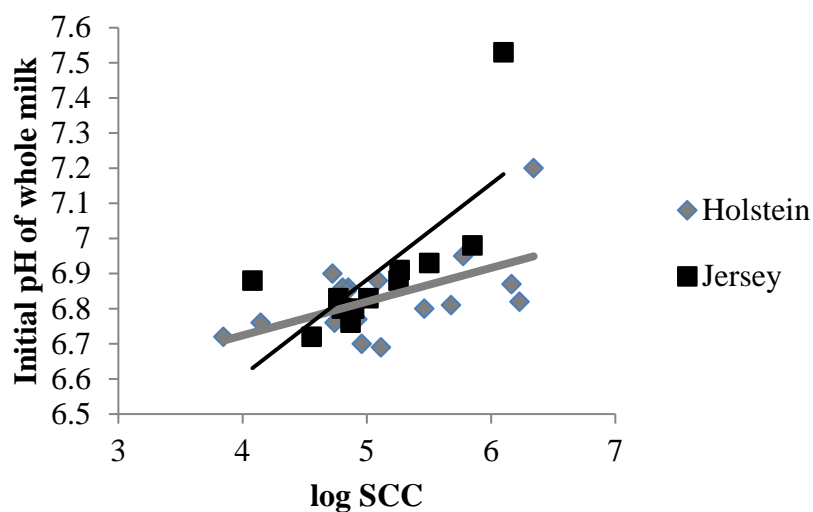


Figure 3. Relationship between somatic cell count and pH of whole milk sample ($P<0.001$).

T_{max} Analysis

A Pearson Correlation value was obtained for each of the following variables and their relationship to T_{max} : DIM, log SCC, initial pH, and fat and protein of the centrifuged milk samples. The various correlation coefficients and their corresponding P-values are shown in Table 3. Of the tested factors, only DIM ($P= 0.004$) had a significant correlation to T_{max} . The variable T_{2min} was highly correlated to T_{max} ($P<0.001$).

Variable	Pearson Correlation Coefficient	
	Tmax	T2min
Tmax	-	0.992***
Log SCC	0.210	0.082
Days in Milk	0.497**	0.316
Initial pH	0.228	-0.038
Centrifuged Milk %Fat	0.184	0.180
Centrifuged Milk %Protein	-0.085	0.000

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$

Table 3. Calculated Pearson correlation coefficients and their corresponding p-values assessing the relationship of log somatic cell count, days in milk, initial pH, and skimmed milk fat and protein composition to T_{max} (n=31) and T_{2min} (n=26).

A multivariate stepwise linear regression model was generated to assess the strengths of breed, skim milk protein and fat composition, DIM, log SCC, parity, and pH of whole milk sample at predicting T_{max} (Table 4). The results of the final linear regression model are shown in Table 4. Breed, initial pH and protein content in the centrifuged milk were found to be significantly associated. On average, T_{max} for Jersey cow milk was 3.63 minutes shorter than Holstein cow milk. For each unit increase in initial pH, T_{max}

increased 11.74 minutes. Interestingly, for every unit increase in protein composition T_{\max} increased by 8.03 minutes.

Predictor	Coefficient	SE Coefficient	P-Value
Constant	-90.55	31.93	0.009
Breed	-3.63	0.92	0.001
Skim Milk Protein	8.03	3.15	0.017
Initial pH	11.74	3.42	0.002

Table 4. Linear regression analysis with breed, skim milk protein, and initial pH as predictors of T_{\max} .

A student's t-test was performed to compare differences in T_{\max} between cows with an SCC level below 200,000 cells/mL and cows with SCC level above 200,000 cells per mL. The results of this test were insignificant ($P= 0.831$), suggesting that there is no evidence that SCC influences T_{\max} . A student's t-test was also done to determine if breed was a significant indicator of T_{\max} . The mean T_{\max} for Jersey milk ($n=13$) was 12.54 minutes and the mean T_{\max} for Holstein milk ($n=18$) was 14.43 minutes. There was strong evidence to suggest a difference in means ($P= 0.034$) between the two breeds. When a student's t-test was used to compare Holstein milk with SCC level below 200,000 cells/mL and Jersey milk with SCC below 200,000 cells/mL, the difference in T_{\max} was significant ($P= 0.006$), suggesting that both breed and SCC should be considered when comparing coagulation times.

Comparison	Groups Compared	P-Value
T _{max}	Holstein vs. Jersey	0.034
	Holstein low SCC vs. Jersey low SCC ^a	0.006
	Low SCC vs. High SCC ^a	0.831
pH	pH low SCC vs. pH high SCC ^a	0.057

^aLow SCC = <200,000 cells per mL; High SCC = >200,000 cells/mL

Table 5. Results of student's t-test comparing T_{max} values obtained for Holstein and Jersey milk, low somatic cell count Holstein and Jersey milk, low and high somatic cell count for both Holstein and Jersey, and differences in pH between low and high somatic cell count milk.

T_{2min} Analysis

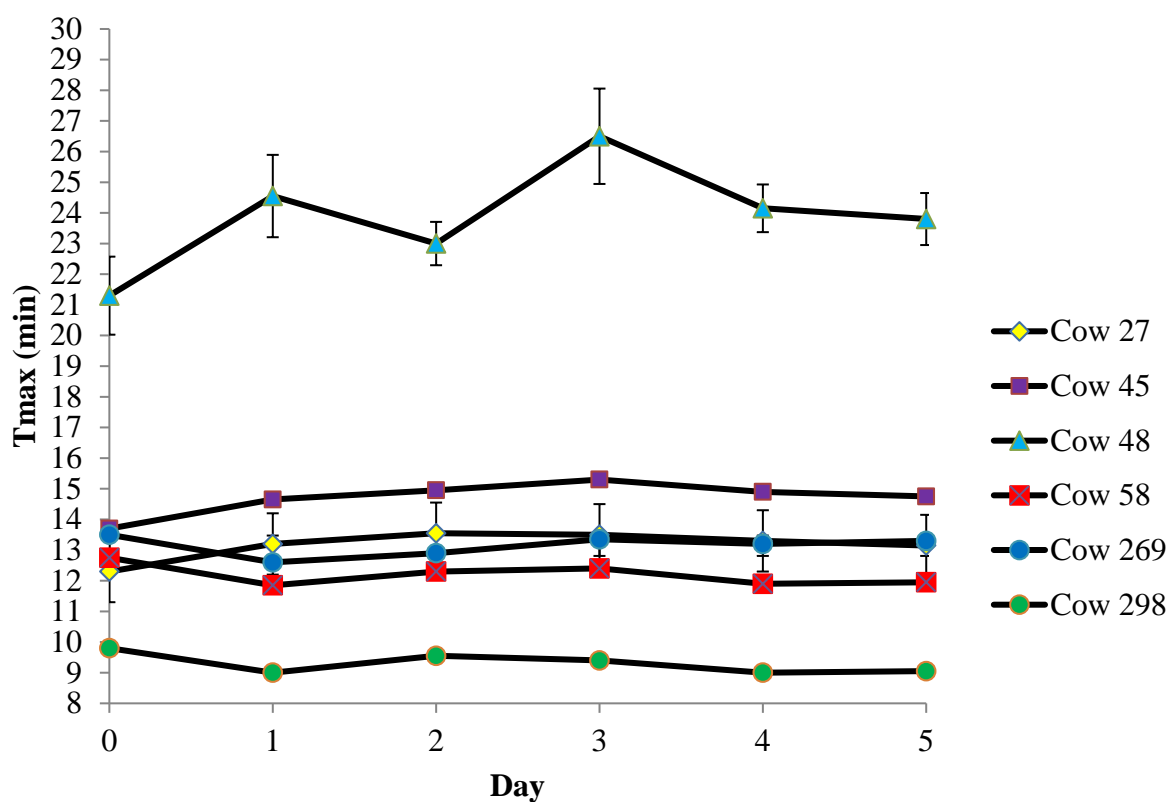
T_{2min} was also investigated to determine any relationship between the gel forming process and the milk composition. Of the 31 cow samples tested, only 26 had T_{2min} data as determined by the CoAguLab. Pearson correlation coefficients for T_{2min} and log SCC, DIM, and fat and protein composition of the centrifuged milk are shown in Table 3. T_{2min} was highly correlated to T_{max} R²=0.992, confirming that coagulation time and initiation of the gel firming process are related.

A student's t-test was done to analyze differences in T_{2min} between breeds. There was some evidence to suggest that the T_{2min} of Holstein milk was between 0.591 and 3.918 minutes longer than that of Jersey milk (p=0.010).

Effect of Milk Age on Coagulation Rate

To determine if the age of the milk influenced coagulation rate, milk was collected from six cows, two Jersey and four Holstein, and held for 0-5 days prior to laboratory analysis. With the exception of one cow, the samples had low variances in coagulation time throughout the five days, averaging between 0.3 and 0.5 minutes. There were no observable patterns in coagulation rates for any of the six cows over the five days. Figure 4 shows a scatter plot of the T_{\max} values obtained throughout the five day analysis.

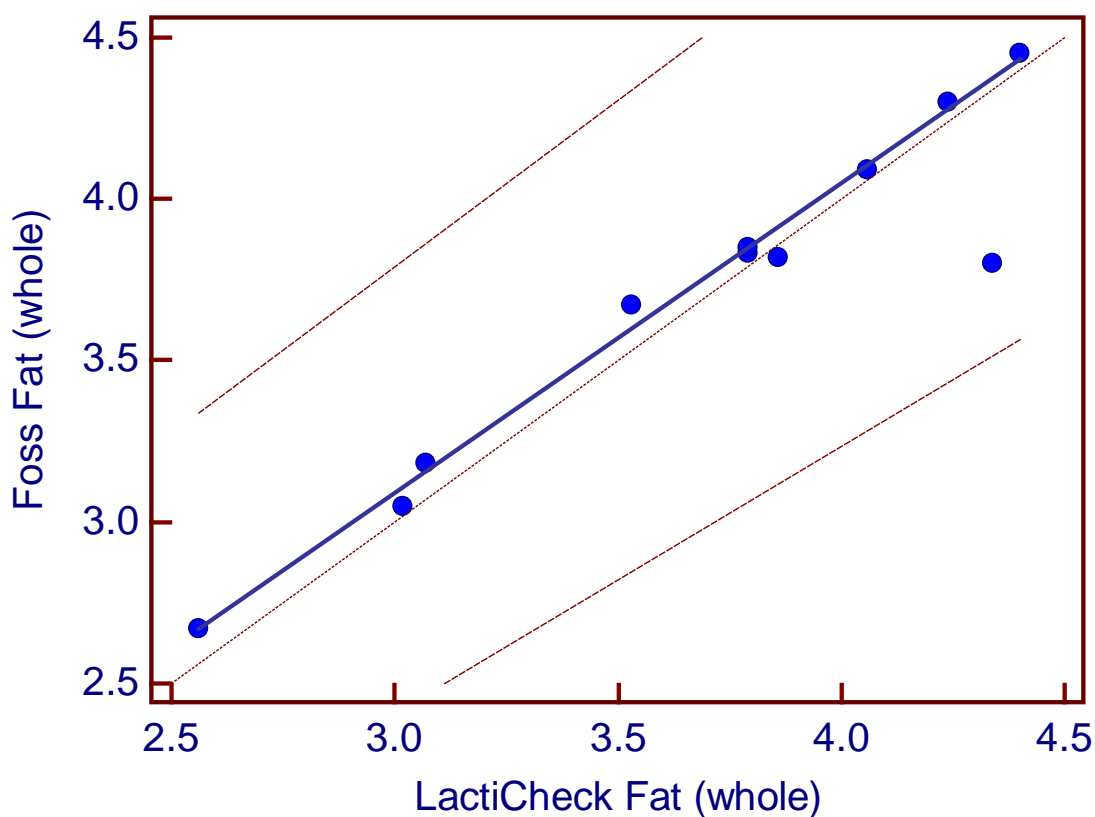
Figure 4: Comparison of T_{\max} values for milk samples from (n=6) individual cows over a five day period. Milk collected from each cow was tested daily for 6 consecutive days to assess the impact of the age of the milk (0 to 5 days) on coagulation rate.



Comparison of LactiCheck and Foss Compositional Analysis

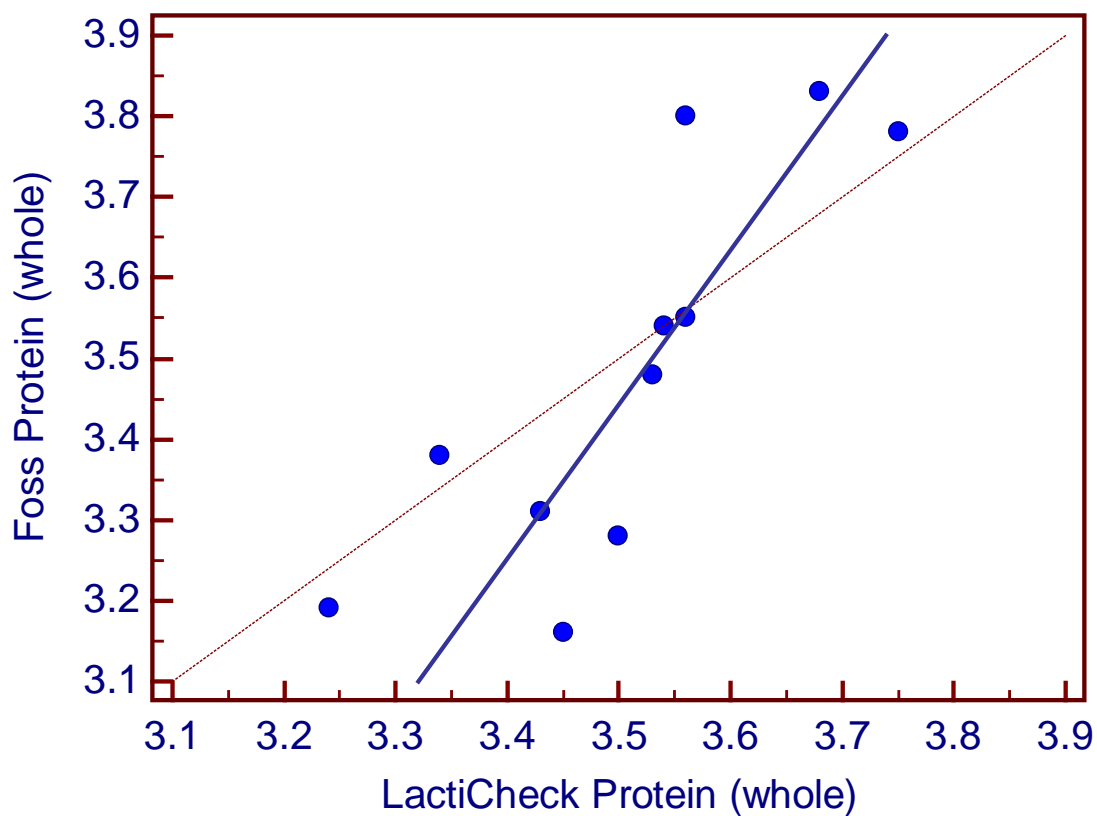
A Passing-Bablok regression analysis was used to determine if the discrepancies in fat and protein composition in the milk samples measured by two different methods were significant. Because two weeks of the data were missing for the fat and protein compositional analysis by the Foss method, only 11 samples could be compared. There was no evidence to suggest a difference in fat values determined by either method (Figure 5).

Figure 5. Comparison of fat composition of whole milk as determined by the Foss and LactiCheck methods. The difference in percent milk fat values obtained by the two methods was not significant.



However there was strong evidence to suggest that the two methods did not correlate well in measuring protein content of whole milk. The deviation from linearity was significant ($P < 0.01$), suggesting that the difference in protein values obtained between the two methods is significant. The LactiCheck technique tended to underestimate protein composition, and should therefore be adjusted by 1.00 to 3.56 units to match the AOAC approved method (Foss).

Figure 6. Comparison of the protein composition of (n=11) whole milk samples as determined by the Foss and LactiCheck compositional analysis methods. According to the Passing-Bablok analysis, protein values obtained from the LactiCheck method should be adjusted between 1.00 and 3.56 units to match the AOAC approved method.



DISCUSSION

Despite the numerous studies examining the influence of DIM, breed, parity, and SCC level on milk coagulation rate, there remains the need for novel methods to resolve the contradicting results obtained. While *Bastian et al.* (1991), *Wedholm et al.* (2006), and *DeMarchi et al.* (2007) did not find a correlation between SCC level and coagulation rate, *Politis et al.* (1988) and *Ali et al.* (1980) found that SCC prolonged coagulation time and weakened the strength of the curd. In addition, *Bastian et al.* (1991) did not find any correlations between parity and MCP, while *Ikonen et al.* (2004) determined that curd firmness decreases with increasing parity.

As the industry moves toward the use of installing on-line sensors to monitor and more accurately predict the rate of coagulation in cheese production, the abilities of such on-line sensors to detect how differences in milk composition effect milk coagulation should also be studied. The use of automated cheese processing equipment would be advantageous because current methods rely solely on the expertise of the cheese maker, which has implications in product uniformity. Aside from the quality control aspects, namely controlling moisture content in the final product, there is also a significant financial loss associated with cutting the cheese curd too early or too late in the processing. It would be advantageous for the cheese maker to know exactly when to cut the curd so as to maximize cheese yield and minimize losses resulting from excessive whey loss. Therefore, the introduction of on-line sensors have the potential to allow cheese makers to maximize their yield, and to better ensure product uniformity.

In addition, it would be beneficial to determine which milk will optimize yield for cheese making based on the cow's age, breed, SCC level, parity, and lactation stage. If for example, jersey cows with low SCC levels that are in mid-lactation produce milk that will yield the highest amount of cheddar, cheese makers could potentially designate or reserve that milk for cheese. The results of this study suggest that diffuse reflectance could potentially help to identify the specific milk compositional guidelines for optimizing cheese yield. The methods utilized in this study were in agreement with several other methodologies with respect to the lack of a correlation between SCC level and RCT, the finding that breed significantly impacts RCT, and that parity does not impact RCT. The results obtained in this study suggest the potential for the use of diffuse reflectance to identify which cow factors should be targeted to better optimize cheese yield.

With the recent defeat of the motion to reduce the legal SCC limit to 400,000 cells per mL (National Milk Producers Federation News Release, 2011) more research is needed to better define the relationship between SCC level and product quality. The results of this study indicate that SCC level does not directly affect coagulation time. This finding is in agreement with others who also found that SCC was not a significant predictor of milk coagulation time (Wedholm *et al.*, 2006; Bastian *et al.*, 1991; De Marchi *et al.*, 2007). However, coagulation time is not the only factor that should be considered. The impact of SCC on sensory aspects has also been studied (Mazal, G. *et al.*, 2007; Barbano, DM *et al.*, 1991; Ma *et al.*, 2000). Other negative quality attributes such as increased moisture content and presence of off-flavors as a result of increased lipolysis are also correlated to elevated SCC levels (Mazal, G. *et al.*, 2007; Barbano, DM *et al.*, 1991; Ma

et al., 2000). Gel strength is also an important indicator of raw material quality with regards to cheese milks. Although gel strength was not directly examined in this study, several papers have indicated that high SCC levels have a negative impact on coagulum strength and yield (Klei *et al.*, 1998; Barbano, DM *et al.*, 1991). Because the techniques utilized in this study only allowed for analysis of milk coagulation time, impact of SCC on coagulum strength and sensory could not be determined. However, it is the author's opinion that the overall impact of high SCC levels should not be restricted to milk coagulation time, but should rather be considered with respect to alterations in moisture content and the presence of negative sensory attributes as well.

Fat and protein composition of cheese milks has many influences on milk coagulation time and yield. Because most of the previous studies that examined milk coagulation properties from individual cows used whole milk for the analysis, the impacts of SCC were most likely influenced by the fat and protein composition. In the original study design, whole milk samples were analyzed. However, the range in % fat was too varied between cows (<2% fat to >5% fat) to distinguish whether the SCC level was causing the variation or the fat composition of the milk. The analysis of skimmed milk samples allowed for a more appropriate determination of how the SCC level altered the coagulation properties of the resident proteins. The results from this analysis suggest that increases in protein prolonged T_{max} , suggesting that the increased protein fraction was non-casein protein which would prolong coagulation by interfering with the formation of the protein network.

It is well documented that milk coagulation is pH dependent, and that cow milk with lower pH favors faster coagulation times and higher curd firmness (Lucey, 2002; Esteves

et al., 2003). However, because the pH of all milk samples were adjusted to pH 6.55, this effect was negated. The results of the linear regression analysis suggest that the initial pH was still a significant influence of coagulation rate. Milks that possessed a higher initial pH, on average, had a prolonged T_{max} . The relationship between elevated pH of milk with high SCC content is likely due to the presence of other protein fractions including lactoferrin, plasmin, and immunoglobulins (Harmon, 1994). This could explain why the initial pH of the milk was a significant predictor of milk coagulation rate in this study, even though the pH of the milk samples were all adjusted to 6.55. This suggests that the presence of foreign proteins in the milk, such as immune proteins that enter the mammary gland during a mastitis infection, could explain why milks with high initial pH values possessed prolonged coagulation rates.

The evaluation of milk coagulation time with respect to breed, parity and DIM was done to assert how the cow's milk coagulation properties vary during her lactation. Several studies have examined the milk coagulation properties of different breeds, but these samples all used whole milk for their analysis (Okigbo *et al.*, 1985; De Marchi *et al.*, 2007). After fat removal, breed was still found to be a significant indicator of coagulation time, with Jersey milk coagulating faster than Holstein milk. This result is in agreement with Okigbo *et al.* (1985) who found that Jersey milk coagulated faster on average, regardless of SCC level, than Holstein milk. DIM was also found to be significant in this study, possibly suggesting that protein composition changes during lactation. This is consistent with *Bobe et al.* (1999), who found parity, season, and lactation stage to significant influences in relative frequencies of milk protein composition (Bobe *et al.*, 1999).

CONCLUSION

Overall, milk coagulation is a complex system that is influenced by numerous factors. Although a number of studies have examined how variations in breed, parity, DIM, SCC levels, and pH effect milk coagulation rate, few studies have come to the same conclusions. Often times, studies done on the milk coagulation from individual cow samples used whole milk for their analysis. This introduces several confounding variables, which may have a significant impact on the ability to identify how specific influences may affect milk's ability to coagulate. Milk coagulation rate represents just a fraction of the milk quality equation. Because milk from individual cows varies in protein composition as influenced by breed, age, parity, and DIM, future research efforts should focus on testing milk from individual cows over an extended amount of time to determine if SCC truly impacts milk coagulation time.

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