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DEVELOPMENT OF A NONCONTACT SENSOR FOR MONITORING MILK COAGULATION AND CUTTING TIME PREDICTION IN CHEESE MAKING

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THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
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By

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Lexington, Kentucky

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ABSTRACT OF THESIS

DEVELOPMENT OF A NONCONTACT SENSOR FOR MONITORING MILK COAGULATION AND CUTTING TIME PREDICTION IN CHEESE MAKING

Cheese products are manufactured more consistently and with better quality if the curd cutting time can be consistently selected. An optical sensor that accurately predicts cutting time has been developed for large cheese vats, but the initial cost of these sensors makes them uneconomical for small artisan cheese manufacturers. The small artisan cheese vats require an inexpensive sensor technology that can be implemented simply. The initial cost of purchasing a sensor and installing these sensors plus the need for a computational program for implementing the algorithm make this technology excessively expensive for these smaller cheese manufacturers. The objective of this research was to develop a simpler sensor technology that can be implemented inexpensively by artisan cheese makers. A prototype sensor has been developed and shown to measure the coagulation of milk in initial experiments. This sensor uses the same concepts for estimating cutting time and much of the same technology as the light backscatter technology; however, it is considerably more cost effective than a light backscatter sensor welded permanently into a vat. The results will show the unique and novel design and characterize its performance on unhomogenized milk.

KEYWORDS: Cutting Time, Cheese, Milk, Coagulation, Backscatter

Molly Craft-Jenkins

July 8, 2012

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CHAPTER 1: INTRODUCTION

Cheese is a staple in the diet of many cultures around the world. Though the process of cheese making is a highly standardized procedure during which milk is converted into various types of cheese through an enzymatic destabilization of casein proteins to form curd, the scale of this process varies widely from producer to producer. Cheese can be manufactured in large commercial dairy plants in tanks that fill an entire room, artfully crafted in small artisan vats, or made in a large pot in a residential garage. No matter what the operation looks like, however, accurate cutting time determination is extremely important to both the quality and the quantity of cheese being produced.

The *Literature Review* of this thesis will further discuss the importance of accurate cutting time determination and various methods that have been developed not only to *determine* cutting time, but to *predict* it. An optical system that can accurately predict cutting time has been made commercially available. However, this system, including the sensor hardware, installation, and the software required to run it, is quite expensive, and though large cheese making operations can spread the cost over a large production volume, this is not the case for smaller cheese makers who cannot justify a costly cutting time prediction technology for limited use on smaller quantities. Therefore, artisan cheese makers must implement less accurate cutting time determination methods, which can lead to a less reliable quality and quantity of cheese.

In this thesis, a new sensor was developed for predicting cutting time in the cheese making process. This sensor, which implements many of the same principles of the sensor that is already available, is far less costly, as it was designed to be mounted *above* the milk instead of drilled in the side of the vat. This allows the possibility for the sensor to be portable, giving the producer the ability to use it on more than one vat. In addition, because the sensor never

actually comes in contact with the milk, maintenance and cleaning are much easier. This sensor was developed to be marketed toward smaller artisan cheese makers who work with several small vats instead of a few large vats, so that they could meet the quality standards set by larger manufacturers without the cost involved in installing a more expensive sensor.

In this work, the *Literature Review* outlines the cheese-making process and gives a history of various methods that have been developed for cutting time determination and coagulation monitoring during cheese-making, highlighting the use of optical sensors for this application and the properties of light that make that possible. The *Materials and Methods* section gives a detailed outline of the experimental procedure for each of the four experiments performed in the development of a noncontact sensor, as well as the components necessary complete each experiment. In the *Results and Discussion* section, the outcome of each experiment is presented graphically, and the statistical analysis of the experiments performed to determine the angle at which the sensor should be mounted are discussed. Finally, the *Conclusions* section discusses the validity of each experimental outcome and suggests future work necessary to continue the development and implementation of a noncontact sensor in a commercial dairy plant.

CHAPTER 2: LITERATURE REVIEW

2.1 Milk Structure

Milk is composed of a complex mixture of water, fat, protein, lactose, and other vitamins and minerals. The breakdown of this mixture in the average cow's milk is 87% water and 13% solids. *Table 2.1.1* (Jenness et al., 1999) shows the average percentage of various constituents in natural bovine milk.

Table 2.1.1 Average Composition of Natural Bovine Milk

Component	Average Percentages
Water	86.6
Fat	4.1
Protein	3.6
Lactose	5.0
Ash	0.7

Proteins make up 3.6% of the total composition of bovine milk. These milk proteins can be separated into two categories: casein and whey proteins. Caseins make up 80% of these proteins, and are the primary building blocks of curd, which is used to make cheese. Though whey proteins make up the other 20%, in the instance of cheese making, whey has been historically discarded. However, modern cheese makers now have the ability to isolate concentrated whey proteins for use in other processes.

Though its three-dimensional structures are not known, the primary structure of casein shows that these proteins distribute residues in separate polar and hydrophobic regions. This structure suggests the formation of distinct polar and hydrophobic areas, resulting in an amphipathic shape. One of the defining characteristics of caseins is their formation of the casein micelle through interactions with other caseins in the presence of calcium. This basic structure is

illustrated in *Figure 2.1.1*, which shows how caseins align to form a micelle with a hydrophilic outer shell and a hydrophobic core (Phadungath, 2005).

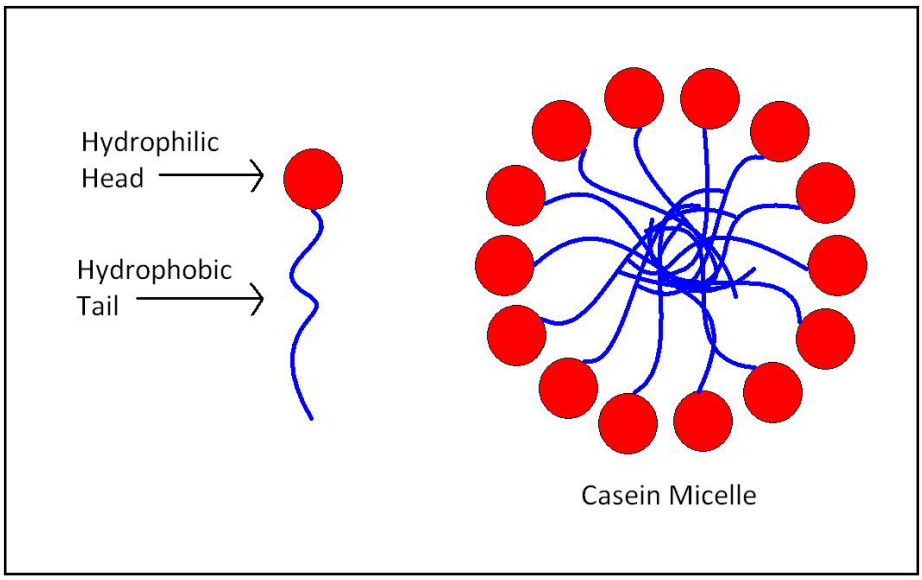


Figure 2.1.1. Basic model of casein association to form micelles (Phadungath, 2005).

However, a more popular model for the formation of micelles from individual caseins illustrates the theory proposed by Pieter Walstra in 1984, which claims that several smaller spherical "submicelles" associate into a larger spherical structure. In this case, there are two types of submicelle, the hydrophobic spheres of individual caseins that form the inner core of the micelle, and a hydrophilicclusters of individual caseins that form the micelle's outer layer. Furthermore, in this model, a C-terminal region of κ-casein, which is strongly hydrophilic, coats the surface of the submicelles on the outer layer of the micelle. The C-terminal end extends from the micelle surface, giving the appearance of a "hairy" layer, which prevents further aggregation of the submicelles, *Figure 2.1.2*.

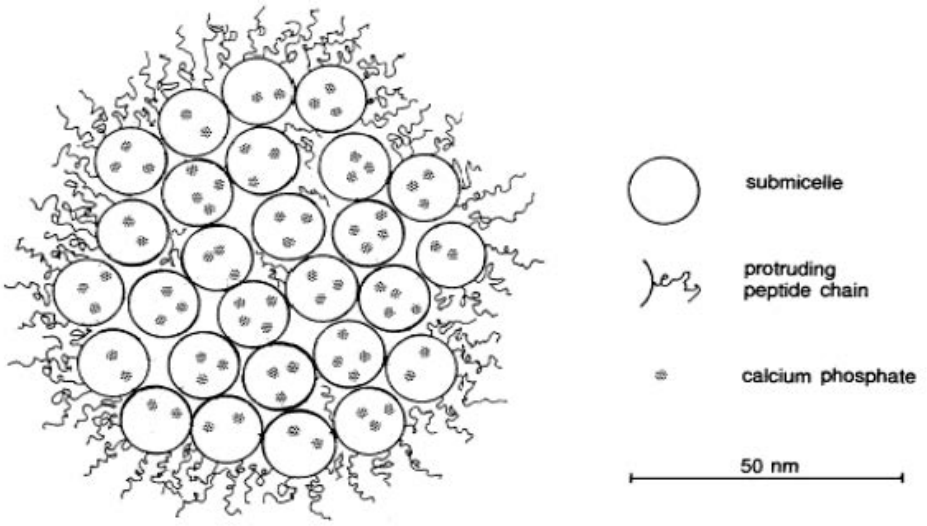


Figure 2.1.2. The structure of casein micelle in the sub-micelles model showing the protruding C-terminal parts of κ-casein as proposed by Walstra (Phundagath, 2005).

The above model was adapted by Dalgleish et al. (2004) to include, in addition to the submicelles, hydrophobic core, and κ-CN outer layer, tunnels running throughout the micelle from the hydrophilic κ-CN existing on the submicelles in the core through which molecules can pass. In *Figure 2.1.3*, an electron micrograph of the casein is pictured.

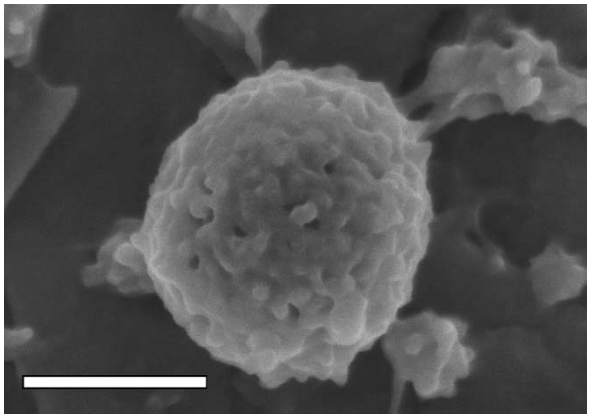


Figure 2.1.3. An electron micrograph of individual casein micelle (Scale = 200nm) (Dalgleish et al., 2004).

Casein micelles are extremely stable in the presence of many environmental variations. However, destabilization and aggregation of these proteins is the goal of the cheese-making process.

2.2 The Process of Cheese Making

The largest influences on the cheese-making process are the characteristics of the milk that is used in the process. The milk proteins, in particular, are very important to the quality of the cheese, the outcome of which is highly dependent on the structure and interactions of these proteins. Changes in milk composition can influence the taste and texture of cheese in a variety of ways. To that end, milk composition in the cheese-making process is highly standardized to achieve uniform fat-to-protein ratios depending on the specific type of milk desired. *Table 2.2.1* below lists the fat and protein content of the most common types of milk.

Table 2.2.1 Fat and protein content in various milk concentrations (Dairy Council, 2012).

Milk Type	Protein %	Fat %
Whole	3.3	3.9
Semi-Skimmed (2%)	3.5	1.8
Skim	3.5	0.3

Whole, skim, and 2% milk can all be used to make cheese, but the types of cheese that each can produce contrast as much as the milk from which they are made. Whole, or full-fat, milk is used to make hard cheeses, such as Swiss and cheddar. Skim, or nonfat, milk is used in the making of drier cheeses, such as parmesan (Dairy Council, 2012). After the milk is determined to have the correct ratio of constituents, it is then pasteurized, or heated in order to destroy heat-labile organisms.

After pasteurization, the milk is ready to begin the formation of curd. The milk is placed in a vat (*Figure 2.2.1*), and calcium chloride can be added to assist in coagulation (the aggregation of casein micelles as explained above).

Figure 2.2.1. Milk in a commercial cheese-making vat.

Next, a lactic acid bacteria culture, along with an enzyme (chymosin or rennet, natural enzymes found in the stomach of most mammals), are added to the milk in order to further aid the coagulation process. Through the fermentation of lactose in the milk, the bacterium produces lactic acid and the pH of the milk begins to drop, thus destabilizing the casein micelles. The polar domains of the calcium-sensitive caseins carry a large net negative charge at the pH of milk, as shown in *Figure 2.2.2*, which represents a net negative charge for the entire molecule. Therefore, the properties of this protein are sensitive to ionic strength.

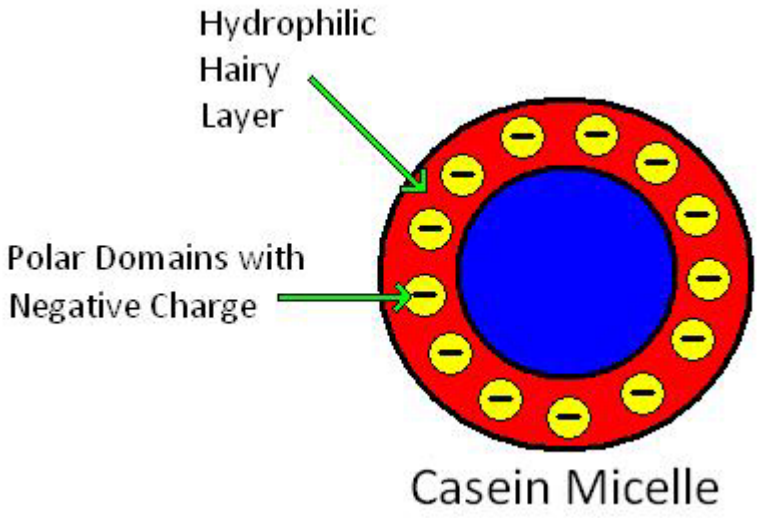


Figure 2.2.2. Casein micelle has been stabilized at the pH of milk by the negative charges of the polarized casein submicelles.

The process of coagulation occurs as the casein micelle begins to destabilize either as a result of the decreased pH neutralizing its negative charge, or the enzymatic removal of protein strands that protrude on the surface of the casein micelle by rennet. Either way, in the absence of this stabilizing layer, casein micelles begin to aggregate with one another to form a gel. As this gel takes shape, the dehydration of the aggregated proteins forms the curd. Coagulation is complete when a firm gel has been formed from the aggregated proteins. This point is known as the “cut time”, or the stage at which the gel must be cut in order to separate the curd from the liquid whey. When the gel is cut, it then undergoes syneresis, a process by which it expels the liquid whey proteins. When syneresis is completed, the final product is curd particles suspended in liquid whey (*Figure 2.2.3).*

Figure 2.2.3. The beginning of syneresis, shortly after the gel has been cut to release the liquid whey proteins.

After syneresis the whey is drained off and either discarded, or isolated and concentrated to be used in other processes. The curd goes on to become any one of a variety of cheese products. The curd may be pressed and molded (in the case of hard cheeses such as parmesan or cheddar), or stretched (mozzarella) into a desired shape. Most cheeses then go through a ripening, or aging process, during which they develop their unique flavor or texture through a variety of biochemical processes.

2.3 Optical Properties of Milk

The interaction of light particles with particles of matter, after which the light particles may change direction or experience a partial loss or gain of energy is known as “light scattering”. Observations of this phenomenon are usually made of the intensity of the scattered light as a function of the angle of scattering relative to the forward direction (Meyer-Arendt, 1995). This intensity can vary based on the materials with which the light is interacting, thus the interpretation of light scatter has many applications.

One such application is in the cheese-making process, as several optical methods have been successfully developed to monitor the coagulation and predict the cut time of certain cheeses. These applications will be discussed further in the following sections; however, the fundamentals of the optical properties behind these systems are worth noting.

Light scattering due to light interactions with casein micelles and fat globules is apparent, even with the naked eye, as it is what gives milk its white color. The light scatters in all directions off of the micelle, therefore there is little absorption by the protein. In the case of monitoring coagulation or predicting cut time using light scatter, several different factors come into play. First, as stated above, light scatters off of micelles in milk in all directions. However, in the cheese-making process, after addition of an enzyme the micelles begin to denature and aggregate. Light scatter off of denatured micelles is much more intense (*Figure 2.3.1*). Therefore, this property of light interactions can be used to quantify the firmness of the coagulum.

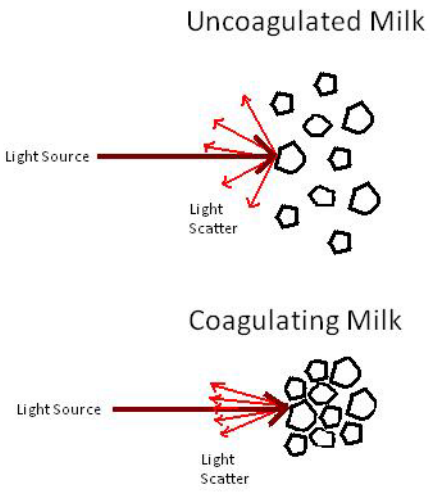


Figure 2.3.1. Illustration of light scatter becoming more focused as milk coagulates

2.4 Methods of Cut Time Determination

During the process of cheese making, one of the most important steps is judging the correct cut time for the coagulant in order to initiate syneresis. Several methods have been developed for testing when the coagulum is ready to cut.

One of the oldest and most widely used methods is the fingertip or knife tip method, in which one inserts a finger, or the tip of a knife into the coagulum and lifts it slightly out of the gel. Cutting stage has been reached when the coagulum shows a clean break and exudes clear whey (*Figure 2.4.1*).

Figure 2.4.1. The fingertip or knife tip method for determining cutting time in coagulated milk.

The above method, however, leaves a wide margin for variability based on the judgment of the tester. As the dairy industry began to grow and cheese manufacturing began to be done in larger production facilities, it became apparent that a more scientific and standardized method for determining cutting time should be developed.

In 1935, Sommer and Matsen developed a visual method for coagulation determination, in which samples of milk with rennet enzyme were placed in a bottle to coagulate. The bottle

was then submerged in water and rotated at a constant rate of 8 RPM (*Figure 2.4.2*). When the film of milk that clings to the inside surface of the bottle showed first signs of graininess or flocculation, it was deemed to have reached the flocculation point. Though this method was the first step toward regulating cut time determination, it still lacked accuracy and was not very practical for use in large-scale cheese processing.

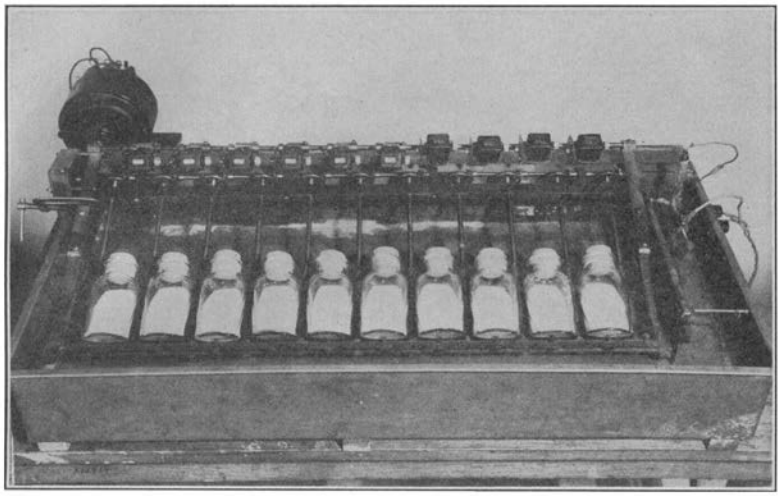


Figure 2.4.2. Equipment used in development of visual method for determining rennet clotting time in milk (Sommer and Matsen, 1935).

In 1964, de Man and Batra proposed a similar, though far less variable, method for cut time determination that used a blood clot timer to define the coagulation point. In this technique, a small sample of milk was placed into the sample cup of a clot timer, rennet was added, and a timer was started. A rotor within the machine stirred the reaction mixture at a constant speed of 50 RPM and as soon as the reaction mixture clotted, the rotor picked up a drop of the mixture and deposited on a set of two electrodes, this closed the detector circuit and stopped the digital timer (*Figure 2.4.3*). The clotting time was then read from the timer dial.

This method, though still not very practical for large-scale operations, provided a highly regulated way to measure the clot time of the coagulant.

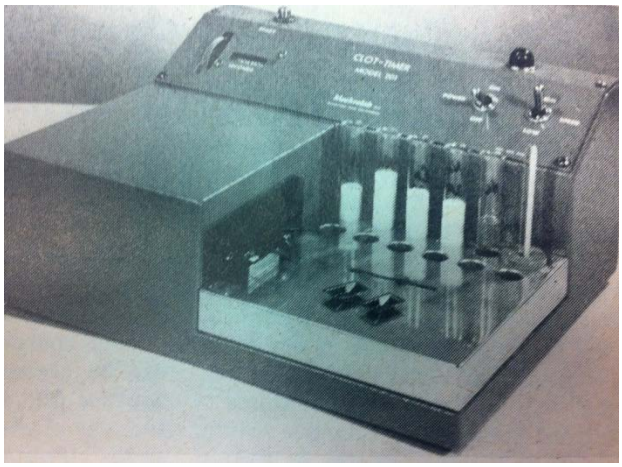


Figure 2.4.3. The Mechrolab 201 Blood Clot Timer used to measure coagulation time in milk (de Man and Batra, 1964).

Several other methods for determining cutting time have been proposed, including using the measurement of viscosity change in milk as an index of coagulation time (Blair and Burnett, 1963). The first proposal of using an optical method for cut time determination was made by Hardy and Fanni in 1981. Their technique involved placing a small, clear cuvette with a renneted milk sample inside on a colormeter and covering it with a black case, then measuring the lightening (L) of the milk as it coagulates. Lightness deviation (ΔL) was calculated using the initial value of the renneted milk. The results of this method are shown in *Figure 2.4.4*.

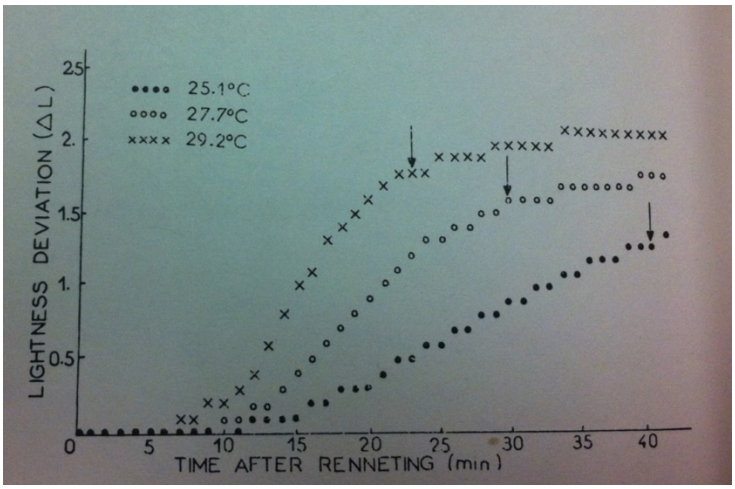


Figure 2.4.4. Lightness deviation of skim milk versus time after renneting. Arrows show visible clotting time (Hardy and Fanni, 1981).

2.5 Optical Methods for Cut Time Prediction

Though the scientific methods for determining cutting time were becoming more accurate, the most widely employed technique remained the fingertip or knife tip method, as the more scientific methods were still difficult to implement in a large-scale milk plant. Also, though the monitoring of coagulation times were becoming more and more precise, a technique had yet to be developed for *predicting* cut time in order to regulate cheese-making on a larger scale.

In 1993, Saputra and Payne et al. developed another optical technique for monitoring enzyme coagulation in milk resulting in a method for predicting enzyme hydrolysis as a model. This method involved placing sample beakers of milk under a window in a gold integrating sphere for spectral data collection. The gold sphere was inside an aluminum probe housing (*Figure 2.5.1)*. Near-infrared light was directed downward into the beaker using a bundle of optical fibers at the top of the sphere, while a second optical fiber bundle on the side of the sphere directed light onto the integrating sphere wall. The reference beam directly illuminated the wall of the integrating sphere, and the sample beam directly illuminated the milk through a

sapphire window. In this configuration, specular reflectance from the glass and milk surface was directed back toward the sample optical fibers while diffuse reflectance (which contained the information from light that interacted with the sample) was scattered into the integrating sphere. Spectral data were recorded as the ratio of the light intensity reflected from the milk sample to that from the reference beam. The diffuse reflectance of the sample was measured by placing the fiber optic probe into a probe shield. An aluminum foil cover was used around the top of the shield to eliminate any stray light. Reflectance scans of each sample were then taken during coagulation (Payne and Saputra et al., 1993).

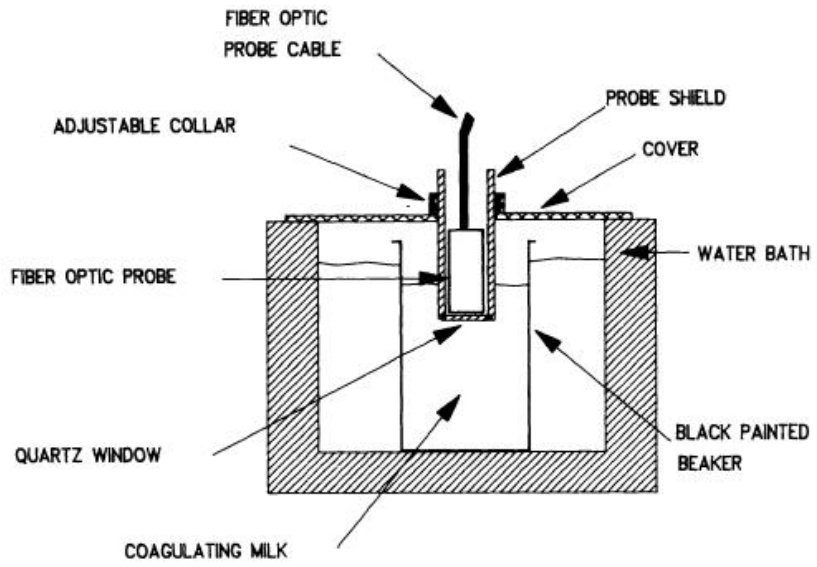


Figure 2.5.1 Optical design for monitoring enzyme coagulation time in milk. (Payne and Suptra et al., 1993)

In 1994, however, Payne et al. proposed a method for coagulation monitoring that could not only be implemented in a large-scale cheese plant, but could accurately predict cutting time. This technique implemented a fiber optic milk coagulation sensor placed inside a stainless steel

tube with a probe tip. The fiber optic cable consisted of two randomly mixed glass fiber bundles. The light from a subminiature lamp was transferred through one fiber bundle to the milk. The light backscatter was transmitted from the milk to the optical detector through the second fiber bundle and a solid state integrated optical detector was used to detect the reflected light from the coagulating milk. A diagram of the apparatus used is shown in *Figure 2.5.3*.

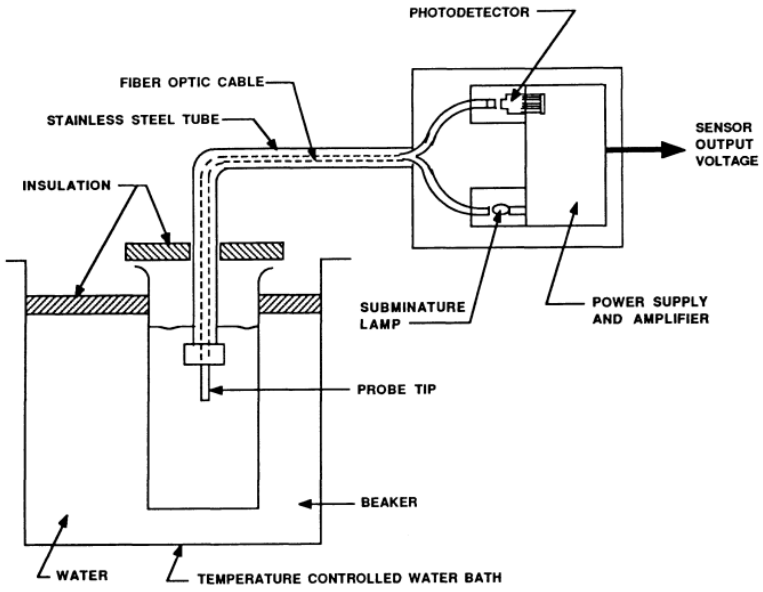


Figure 2.5.3. Fiber optic sensor used to measure diffuse reflectance in coagulating milk (Payne et al. 1994).

Using the results from several system tests, they developed an equation from which cut time can be predicted when t_{max} (or the sigmoidal inflection point of the generated graph of reflectance vs. time *Figure 2.5.4)* is known. This resulted in an equation for predicting cutting time: $t_{cut} = \beta * t_{max}$. This was the first method for extracting information about the status of coagulation that could be used to automate cutting time selection.

In addition, Payne et al. (1998) showed that this method would work consistently over a broad range of conditions (temperature, pH, enzyme concentration, calcium addition level, and

fat content) normally encountered in cheese making. The exception was changes in protein content. Castillo et al. (2002) described the effect of protein content on cutting time selection.

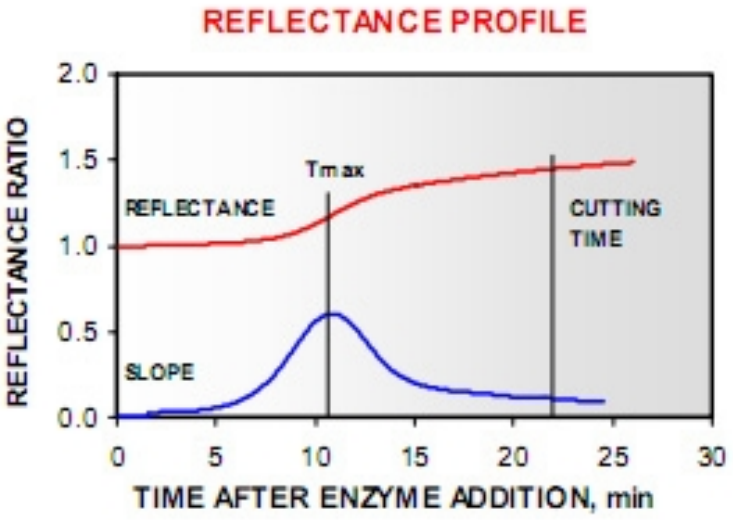


Figure 2.5.4. Typical diffuse reflectance profile of coagulating milk with the induction, sigmoidal, and logarithmic periods; sigmoidal inflection time, t_{max} and observed cut time, t_c indicated (Payne et al. 1994).

A commercial optical sensor for implementing the cutting time selection (CoAguLite sensor manufactured by Reflectronics, Inc., Lexington, KY) has been developed for permanent installation in closed cheese vats. The high throughput of these large cheese making operations spread the cost of the sensor, welding it into the vat, and installing an algorithm in the plant Programmable Logic Controller (PLC) over a large production volume. This is not the case for smaller cheese makers which cannot justify a costly cutting time prediction technology for limited use on smaller quantities. Thus the smaller producers, such as artisan cheese makers, must implement old-fashioned and less precise techniques, such as the fingertip or knife tip method.

2.6 Objective

The objective of this study was to develop a simple and inexpensive optical sensor technology for the small cheese makers. Several concepts were considered but the most attractive was to develop a sensor that could mount above a cheese vat without making contact with the milk. Such a sensor could be easily installed and could be made portable for use on multiple vats thus reducing the cost significantly. The technical challenge was determining a method for obtaining an optical signal from above the milk vat without interference from surface reflectance.The development of this method required three main objectives. The first objective was to determine if a sufficient backscatter signal could be detected with the detector placed above the milk during the coagulation process, and which range of light wavelengths would be the most responsive. The second objective was to determine the specific wavelength and detector combination that would work best for operation of the sensor in a milk plant. The third objective was to find the ideal configuration for the sensor apparatus that would allow for optimal light backscatter detection while maintaining functionality as an inexpensive, easily installed, and portable system.

CHAPTER 3: MATERIALS AND METHODS

3.1 Introduction

Two experiments were initially conceived: first, a series of tests to determine the ideal wavelength for detecting light backscatter off of the surface of a milk vat under ambient light conditions of a cheese manufacturing facility, and second, designing and testing an apparatus for easily mounting a sensor above a milk vat without interfering in the normal operations of the vat. During the design experiments, however, it was discovered that a test was needed to determine the ideal angle for measuring light backscatter.

This objective required four experiments. The first experiment was an evaluation of a concept to determine if a sufficient backscatter signal could be detected with the detector placed above milk during the coagulation process and which light wavelengths were most responsive. After the first experiment was deemed successful, a second experiment was conducted to determine if a ratio of infrared to visible responses would eliminate ambient light interference. The third experiment, the evaluation of the ideal angle at which to mount a backscatter sensor above a milk vat, was determined necessary after initial design tests revealed that specular reflectance could interfere with the signal to a sensor mounted directly above a milk vat. And finally, a fourth test was conducted to verify the results of the third experiment. Together, these experiments were developed to identify the best method and validate the procedures involved in developing a sensor for collecting backscatter data from a vat of coagulating milk while mounted above the surface of the milk.

3.2 Experiment 1 – Evaluation of Sensor Concept

The first test was conducted with the objective of determining if an optical signal similar to that obtained from a vat-mounted signal *(Figure 2.5.3)* could be obtained from the light backscatter above the surface of the coagulating milk.

3.2.1 Experimental Design for Experiment #1

Coagulating milk samples were exposed to light wavelengths from 300 nm to 1100 nm, and the reflectance of each wavelength was recorded throughout the entire coagulation process. These reflectances were then evaluated to determine the effectiveness of this method for gathering data on the coagulation process.

3.2.2 Laboratory Materials for Experiment #1

The milk source used was homogenized two percent milk (Kroger (Winchester Farms Dairy); Winchester, Ky). Two percent milk was chosen because it was an easily-accessible representation of higher fat milks used in cheese-making. A balance and a 2L beaker were used in the preparation of the milk sample. Chy-Max® Extra, a high-quality milk coagulant manufactured by CHR Hansen and made from 100% naturally fermented Chymosin enzyme, was added to the milk sample along with $CaCl_2$ that was prepared in the lab. Addition of $CaCl_2$ expedites the coagulation process by reducing the clotting time and the time it takes the coagulum to become firm enough to cut (Castillo et. al, 2002). A Lauda Ecoline water bath with ±.01°C of accuracy (RE220, Brinkman Instruments, Inc. NY, USA) was used to maintain milk at the optimal temperature for coagulation, 31°C (Sommer and Matsen, 1935).

3.2.3 Optical Materials for Experiment #1

The optical system used was assembled from components of other systems. The apparatus used to simulate a milk vat in a cheese processing facility was the “vat” component of the CoAguLab data acquisition system (CoAguLab; Reflectronics Inc., Lexington, KY), *Figure 3.2.3.1*.

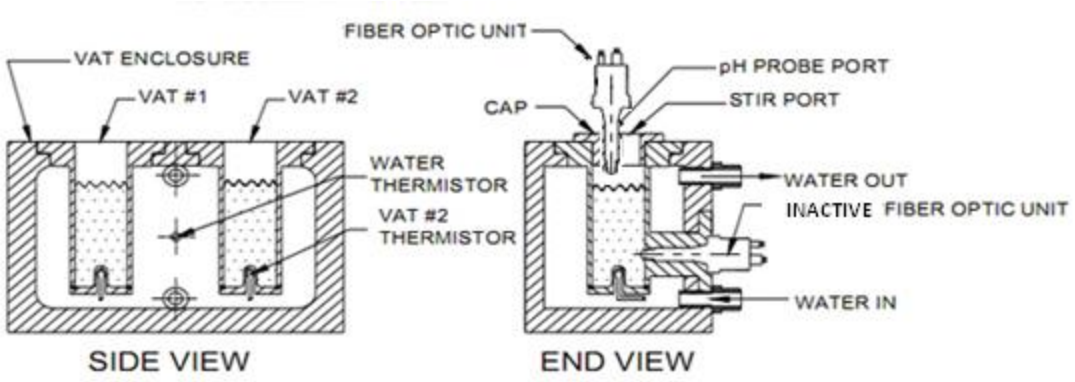


Figure 3.2.3.1. A schematic of the vat component of the CoAguLab data acquisition system (www.reflectronics.com)

The vat shown in *Figure 3.2.3.1* was modified so that the optical probe was positioned above the milk through the pH probe port, and an inactive fiber optic unit was inserted into the fiber optic probe port on the side to prevent leakage. The vat enclosure was connected to a Lauda water bath to maintain sample temperature at 31°C. A tungsten-halogen light source (LS-1; Ocean Optics, Inc. Dunedin, FL, USA) was used for its broad spectrum range (shown in *Figure 3.2.3.2*) and its steady, sustainable signal.

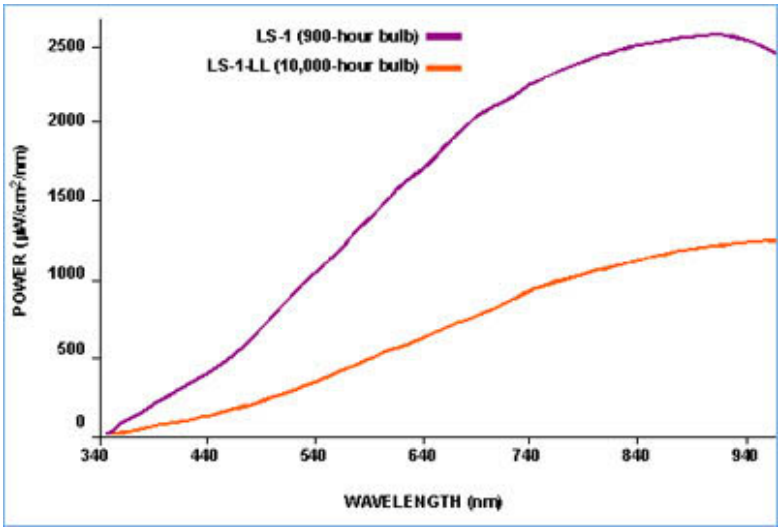


Figure 3.2.3.2. Light Curve for LS-1 light source (www.oceanoptics.com)

In addition, a spectrometer (HR4000 Series; Ocean Optics, Inc. Dunedin, FL, USA) was used to collect backscatter data on the milk as it coagulated. This spectrometer was selected due to its high resolution, wide bandwidth, and user-configured interface. The spectrometer and the tungsten-halogen light source were connected to a fiber optic probe using fiber optic cables that were designed for this purpose. The probe was specifically designed for use with the CoAguLab, and therefore adapted easily to the new configuration. The fiber optic cable that connected to the light source was placed in the far left position of the probe, and a cable connected to the spectrometer was placed in the R1 position. This configuration can be seen in *Figure 3.2.3.3.*

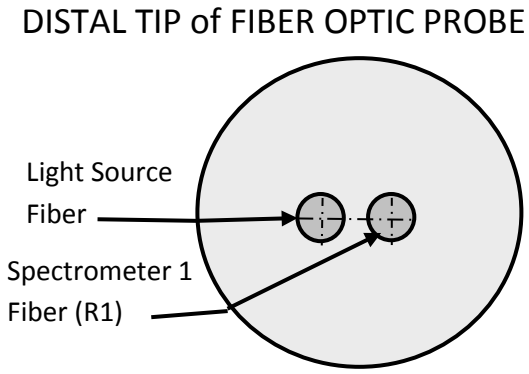


Figure 3.2.3.3. Schematic of distal tip of fiber optic unit illustrating the position of the light source and spectrometer.

When analyzing the data, a ratio of wavelengths within the data collected by the spectrometer was taken. This ratio gives a clearer signal than when only one wavelength is observed, as any interference in the signal due to changes in ambient light disrupt both signals. Therefore, the ratio of the signals to each other remains unchanged. The signal data collected by the spectrometer was charted using the Spectrasuite Spectroscopy Software Platform

(Spectrasuite; Ocean Optics, Inc. Dunedin, FL, USA), which can be configured to the user's specific designs and adjusted for different scanning intervals, based on backscattered light intensity. This program outputs the data collected for each separate wavelength on the broad spectrum as bits/second.

3.2.4 Procedure for Experiment #1

The tungsten-halogen lamp was turned on in order to be operating at peak output by the time it was to be used. A sample of 1 kg of milk was prepared in a beaker and placed in the water bath to heat to 31°C. A measure of 0.74g $CaCl_2$/kg milk was added to the milk sample to aid in coagulation. Meanwhile, electronic zero (the light backscatter picked up by the spectrometer when no light is transmitted through the light source being used) was measured and recorded with the optical data acquisition system to give a reference point for the backscatter data that would be recorded during coagulation. When the milk reached 31°C, a measure of 0.066g Chy-Max/kg milk was added to the milk sample and stirred gently to avoid creating bubbles for 30 seconds. Then 100 mL of the milk sample was poured into Vat #1 of the CoAguLab vat system. Any bubbles that formed on the surface of the sample in the vat were scraped away. The cover was placed over the vat, the fiber optic probe was inserted into the pH probe port, and its tip was positioned 1 cm above the milk. The Spectrasuite software was then activated to collect backscatter data while the milk coagulated for 30 minutes. The test was then repeated once.

3.3 Experiment 2 – Testing two infrared LEDs

3.3.1 Experimental Design for Experiment #2

It was decided based on the results of Experiment #1, that the most promising wavelengths for collecting backscatter information from the surface of coagulating milk were infrared signals in the high 800 nm and high 900 nm ranges. This experiment was designed to

compare the milk coagulation signal responses for a dedicated sensor using an 880 and 970 nm LED and the TSL 257 and TSL 267 (infrared) detectors. These 880 and 970 nm LEDs were selected because they fell within the range where the coagulation response was the highest (*Figure 4.1.2*).

3.3.2 Lab Materials for Experiment #2

The milk used in the second experiment was skim and homogenized two percent milk processed by Winchester Farms Dairy, Winchester, KY. Skim milk was selected because it results in a strong milk coagulation signal. A strong signal was needed to determine the better of the two wavelengths tested. Two percent milk was used to confirm the results found in the skim milk tests with a sample that has a higher fat content that would be similar to milk used in cheese-making. A balance and a 2 L beaker were used in the preparation of the milk sample. To aid in milk coagulation, Chy-Max® Extra was added to the milk sample, along with $CaCl_2$ that was prepared in the lab. A Lauda Ecoline water bath with a reported precision of ± .01°C (RE220, Brinkman Instruments, Inc. NY, USA) was used to maintain milk at 31°C.

3.3.3 Optical Materials for Experiment #2

The optical system used was the CoAguLab data acquisition system (CoAguLab; Reflectronics Inc., Lexington, KY) shown in *Figure 3.3.3.1,* which powered the LED light sources and received the optical signals from light detectors. The system processes the optical information received from the light detectors as voltages and transmits this information to a computer program. The CoAguLab system also has the capability of continuous temperature monitoring. In this experiment, only one of the two available coagulation vats was used, however, because two detectors were being simultaneously recorded. The system was configured such that one detector was wired into the system as “Vat 1” and the other detector

was wired as “Vat 2”. In this way, data from the same milk sample was collected and stored as separate data files.

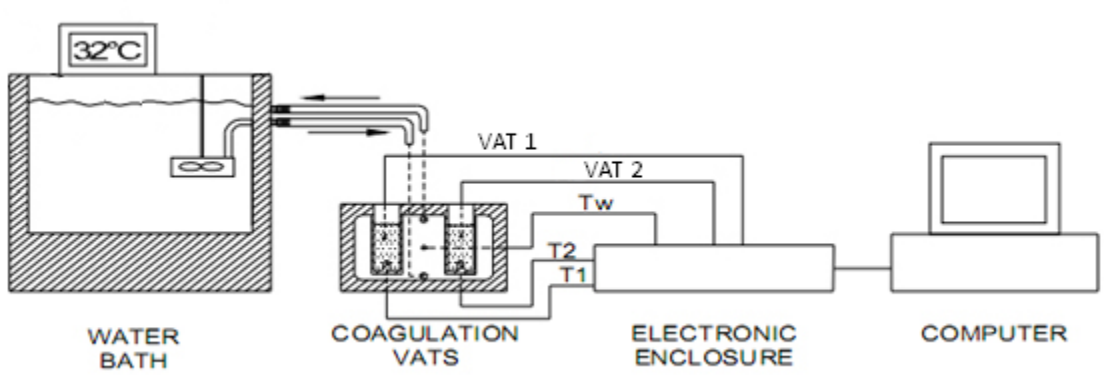


Figure 3.3.3.1. Schematic of the CoAguLab data acquisition system (www.reflectronics.com)

An 880 nm LED (L2791-02, Hamamatsu Corporation; Bridgewater, NJ) and a 970 nm LED (LED970-01; Roithner LaserTechnik; Vienna, Austria) were used as the light sources for Experiment #2. Each of these LEDs had a viewing angle of 20°. There were two light detectors used, both of which read light backscatter and outputted the data as a voltage: the TSL 257 high-sensitivity light-to-voltage converter (TAOS023E; Texas Advanced Optoelectronic Solutions, Plano, Tx), and the TSL 267 high-sensitivity infrared light-to-voltage converter (TAOS033E; Texas Advanced Optoelectronic Solutions, Plano, Tx). Spectral responsivity of the TSL257 (*Figure 3.3.3.2).* Though this detector can read light backscatter over a broad spectrum (from 300 nm to 1100 nm), it is most sensitive to light in the visible range, especially from 500 nm to 800 nm.

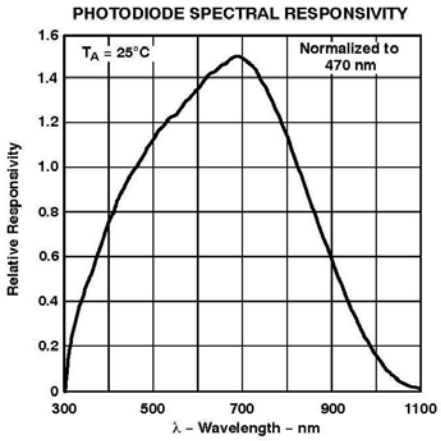


Figure 3.3.3.2. Spectral responsivity of the TSL257 High-Sensitivity Visual Light-to-Voltage Converter. Note: The highest sensitivity is in the range from 500 nm to 800 nm.

Spectral responsivity of the TSL267 (*Figure 3.3.3.3).* This detector can only read light backscatter in the infrared wavelengths (from 800 nm to 1100 nm), but is most sensitive to wavelengths between 850 nm and 1000 nm.

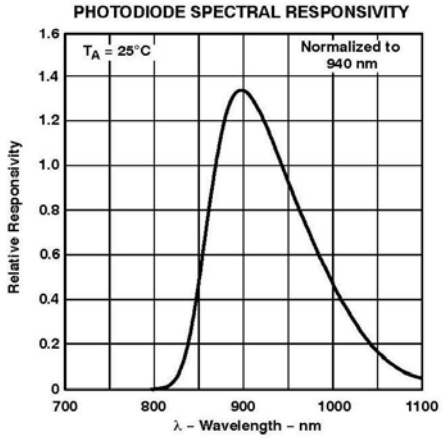


Figure 3.3.3.3. Spectral responsivity of the TSL267 High Sensitivity Infrared Light to Voltage Converter. Note: The highest sensitivity is in the range from 850 nm to 1000 nm.

A prototype of a dedicated sensor was assembled by connecting the LED and detectors to a brass connector base (*Figure 3.3.3.4).* The brass connector base was designed to be positioned above the milk in a CoAguLab vat.

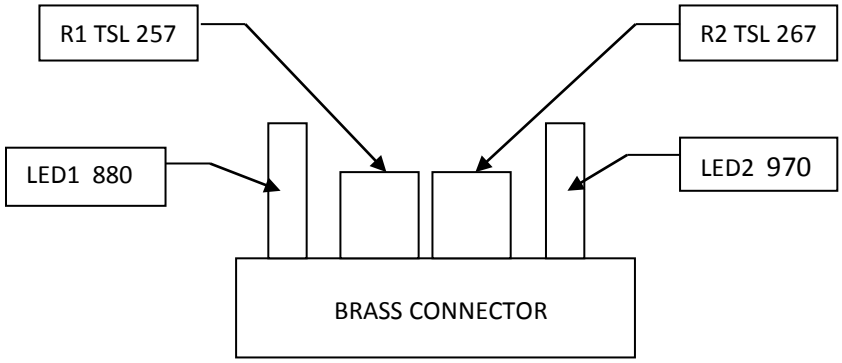


Figure 3.3.3.4. Brass connector base on which LEDs and detectors were mounted.

The CoAguLab data acquisition program (Reflectronics Inc., Lexington, KY) was used to collect and display the light backscatter data. In this experiment, cutting time was arbitrarily selected at 2 times t_{max}. In other words, if t_{max} occurred at 15 minutes, the cutting time would be 30 minutes. The CoAguLab program also stores the light backscatter signals.

3.3.4 Procedure for Experiment #2

The LEDs and detectors were placed in the brass connector base and wired into the CoAguLab Electronic Enclosure. The optical zero was measured by placing a black rubber stopper in front of each detector and collecting backscatter data. Skim milk (100 mL) was placed in Vat 1 of the CoAguLab, and the brass connector base on which the LEDs and detectors were mounted was positioned 1 cm above the surface of the milk. Using the potentiometers in the CoAguLab circuit box, the gain was set for each detector in order to frame the signal within easily analyzed parameters. “Optical zero” was again measured, this time off the surface of the milk in the dark vat. The vat was then emptied and prepared for the actual milk sample. For the

milk sample, 1 kg of skim milk was prepared in a beaker and placed in the water bath to heat to 31°C. A measure of 0.74g $CaCl_2$/kg milk was added to the milk sample to aid in coagulation. When milk reached 31°C, a measure of 0.066g Chy-Max/kg milk was added to the milk sample and stirred gently, to avoid creating bubbles, for 30 seconds. Then 100 mL of the milk sample was poured into Vat 1 of the CoAguLab vat system. Any bubbles that formed on the surface of the sample in the vat were scraped away. The brass connector base was then repositioned over the milk sample at the same point that it was positioned for setting the gain of the detectors. Exactly 1 minute and 30 seconds after the enzyme was first added to the milk, the data collection was started with both LEDs OFF. The LEDs were both turned ON at 2 minutes, and then switched on and off 30 seconds until data collection was stopped at the end of coagulation (2 times t_{max}).The experiment was then repeated with two percent milk.

3.4 Experiment #3 – Angle Determination

In the application of light backscatter in cheese making, it is very important for the light to be able to interact with the casein micelles as they denature and aggregate. However, when light hits the surface of milk at certain angles, it experiences specular reflectance, or the mirror-like reflection of light from a surface, and the light waves simply bounce back without penetrating the surface and interacting with the milk's constituents. It is difficult to determine when this particular phenomenon takes place, as light interactions with liquids such as milk are dependent on the density of the liquid, and as milk coagulates, its density is constantly changing.

3.4.1 Experimental Design for Experiment #3

The objective of this study was to determine the angle of incidence of the illuminating light beam that would maximize the coagulation response. During previous testing it was theorized that specular reflectance from the surface of the milk decreased the optical

coagulation response – the maximum reflectance ratio at t_{max}. Therefore, this test was designed to test several different angles to determine the optimum angle that would maximize the optical coagulation response.

3.4.2 Laboratory Materials for Experiment #3

The milk used was unhomogenized whole milk (UK Dairy; Lexington, KY). Unhomogenized whole milk was chosen due to the fact that the majority of the artisan cheese manufacturers for whom this system will be designed use unhomogenized milk in their cheese making process. The milk was collected and transported in a ten gallon dairy container with a manual agitator. A balance was used in the preparation of the milk, and an open stainless steel metal pot (28.0 cm diameter x 24.0 cm deep) was used as the cheese vat. Chy-Max® Extra, was added to the milk sample, along with $CaCl_2$. A Lauda Ecoline water bath with ±.01°C of precision was used to maintain milk at 31°C.

The milk surface area exposed to the atmosphere in the open stainless steel pot was much larger than in the small vats of the CoAguLab used in the previous two experiments. Consequently, surface cooling was a concern. Both infrared reflectance and enzymatic reaction rate decreases with the surface temperature (based on previous experience). Thus attempts to maximize the optical coagulation response required surface temperature control. A plastic hood was placed over the entire water bath, milk vat, and backscatter detection apparatus to maintain the surface temperature at 31°C. Also, the surface temperature was monitored throughout the test using a Fluke 561 HVACPro Infrared Thermometer (561 HVACPro, Fluke, Everett, WA), which takes surface temperature from a distance, and can be used without disturbing the coagulum.

3.4.3 Optical Materials for Experiment #3

In many commercial milk vats, a stirring system is used at different points during the cheese making process (enzyme addition, coagulum cutting, etc). An optical sensor must avoid interfering with the mixing system. Thus, it was decided that the optical system must operate at a fixed distance of at least 60 cm above the milk. A frame system was designed that would keep the monitoring system at the required height while allowing the sensor and light source to rotate at different fixed angles for testing (*Figure 3.4.3.1 and Figure 3.4.3.2)*. The frame could rotate 30 degrees forward or backward to test different angles of incidence. The exact angle was determined and verified by a protractor affixed to the side of the water bath. The protractor's angles matched the bar of the frame when the frame was aligned correctly.

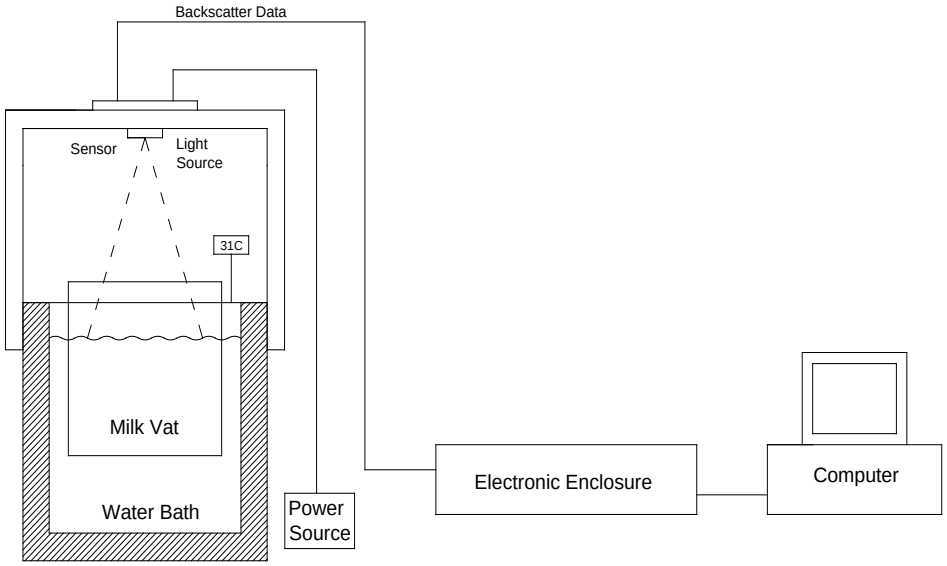


Figure 3.4.3.1. Optical system used in experimentation with rotating frame sensor mount above the milk vat.

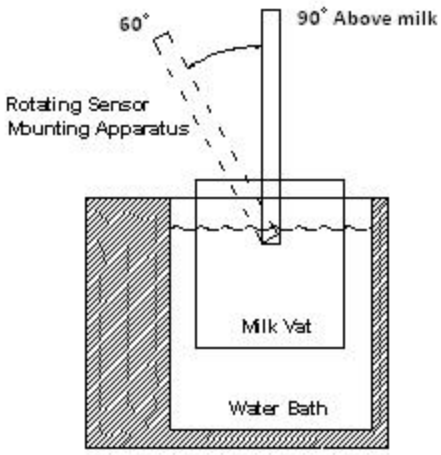


Figure 3.4.3.2. Side view of rotating frame sensor mount above milk vat.

A prototype illumination system was developed by assembling a simple circuit using twenty 970 nm LEDs and a TSL267 high-intensity infrared light-to-voltage converter. The LEDs and the detector were configured on a circuit board as shown in *Figure 3.4.3.3*. This board was fixed to the rotating frame mounted to the sides of the water bath as illustrated in *Figure 3.4.3.1.*

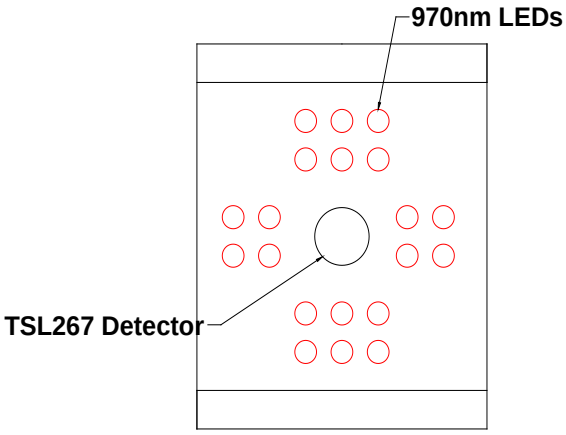


Figure 3.4.3.3. Configuration of LEDs and detector used in experimentation

The circuit for the LEDs was powered by a Hewlett Packard 6236B Triple Output Power Supply (6236B; Hewlett Packard, Palo Alto, Ca). Approximately 1 amp of current at 5 V output was required to power the 20 LEDs. The detector was connected to the CoAguLab electronic enclosure. The CoAguLab (Vat 1 circuit) software was used to record the detector signal during the coagulation sessions (*Figure 3.4.3.1).*

3.4.4 Procedure for Experiment #3

The milk was stirred with the manual agitator in the ten gallon dairy container for 30 seconds. Then a portion of the milk was poured into a two gallon container and stirred with a large spoon for 30 more seconds. A 3750 g sample of milk was then weighed out into the stainless steel cheese vat and placed in the Lauda water bath to heat to 31°C. A measure of 0.74g $CaCl_2$/kg milk was added to the milk sample to aid in coagulation. Meanwhile, the rotating frame was positioned at the desired angle for testing and fixed in place with tape. The plastic cover was then placed over the entire system to ensure even heating of the milk sample. When the surface temperature of the milk sample reached 31°C, an ambient light reading was taken with the LEDs OFF to obtain a reference for the light in the room. The plastic covering was then lifted, and 0.066g Chy-Max/kg milk was added to the milk sample and stirred gently for 30 seconds to avoid creating bubbles. Any bubbles that did form on the surface were scraped away. The plastic covering was then replaced and the sample was allowed to settle for 30 seconds. Then the LEDs were turned ON, and data collection initiated. During data collection, the surface temperature of the coagulating milk sample was taken every 5 minutes to ensure that it remained at 31°C ± 1°C. The data collection was ended at 2 times t_{max}. This test was performed for angles of incidence of 90°(directly above the milk), 80°, 75°, 70°, and 60° with three replications.

3.5 Experiment #4 - Angle Verification

3.5.1 Experimental Design for Experiment #4

A simpler and more direct measurement of the difference between coagulated and non-coagulated milk at different illumination angles was developed to verify the results measured in Experiment 3. A device was built to measure the reflectance change between coagulated and non-coagulated milk at different angles. Both raw unhomogenized and skim milks were tested for light backscatter at the beginning and end stages of coagulation and at various angles and distances. A statistical analysis of this data was then performed to validate the findings of Experiment 3.

3.5.2 Lab Materials for Experiment #4

The milk used was unhomogenized whole milk (UK Dairy; Lexington, Ky) and skim milk (Kroger (Winchester Farms Dairy); Winchester, Ky). These two milk types were chosen to verify the results found in Experiment 3 for both full fat and nonfat milk samples. A balance was used in the preparation of the milk, and large flat pans were used as containers for the milk samples during testing, and a sample of milk with a depth of 3 cm was prepared in each pan. Chy-Max® Extra was added to the milk samples that were coagulated, along with $CaCl_2$. A Lauda Ecoline water bath with ±.01°C of accuracy was used to maintain milk at 31°C, and a Fluke 561 HVACPro Infrared thermometer was used to monitor the surface temperature of the milk throughout the test.

3.5.3 Optical Materials for Experiment #4

This test was performed on a lab bench, and a special apparatus was designed in order to evaluate the signal detection at various angles. The apparatus, shown in *Figure 3.5.3.1,* consisted of a rotating arm suspended between two upright metal rods.

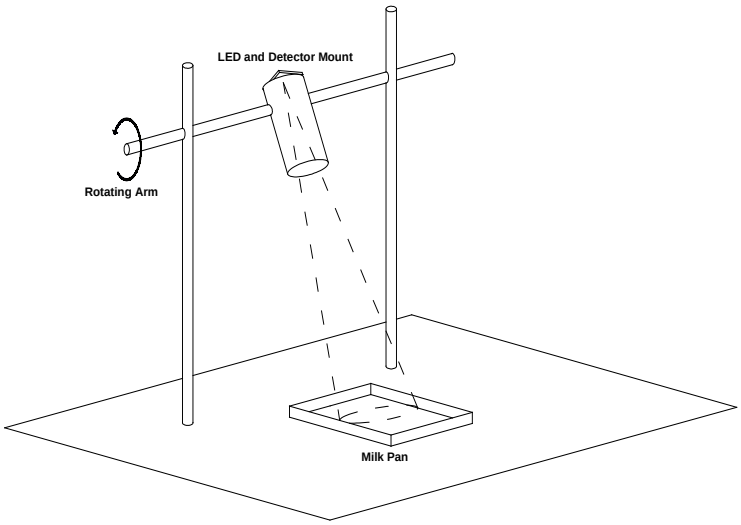


Figure 3.5.3.1. Schematic of apparatus designed to verify angle data found in Experiment 3

The simple LED and detector circuit used in the previous experiment was attached to the rotating arm, and a 3" diameter cylinder of PVC pipe was fixed to the viewing side of the circuit board to focus the light on the pan containing the milk sample. The angles were measured with a protractor, and the milk pans were moved into the line of sight for each angle as the arm rotated. The LED and detector circuit was powered by a Hewlett Packard 6236B Triple Output Power Supply (6236B; Hewlett Packard, Palo Alto, Ca) and wired into the CoAguLab circuit box so that data collected by the light detector could be processed with the CoAguLab software.

3.5.4 Procedure for Experiment #4

Two trays were prepared with 2000 g milk in each. The milk samples were then placed in the water bath and allowed to heat to 31°C. A measure of 0.74g $CaCl_2$/kg milk was added to the milk sample in Tray #1 to aid in coagulation. When the trays reached 31°C, 0.066g Chy-Max/kg milk was added to the milk sample in Tray #1 and stirred gently to avoid creating bubbles for 30 seconds. Any bubbles that did form on the surface were scraped away. Tray #1

was then allowed to coagulate in the water bath for 30 minutes. Tray #2 was moved to the lab bench and placed at 90°, directly beneath the PVC cylinder. The LEDs were turned OFF, and a scan was taken. Then the LEDs were turned ON, and another scan was taken. The tray was moved to 80° from the milk, and the horizontal distance from the base of the apparatus was measured. The rotating arm was adjusted so that the milk was in the viewing range of the detector, scans were taken with the LEDs OFF and ON, and the surface temperature of the sample was taken. This process continued at angles 75°, 70°, and 60°, respectively. Then the scans were repeated on the same sample. When Tray #1 finished coagulating, the same process was performed on it. This test was repeated for both unhomogenized whole milk and skim milk.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Experiment 1 – Evaluation of Sensor Concept

The light backscatter signal for the first test is shown in *Figure 4.1.1*. This graph shows that there is an increase in signal strength in the higher wavelengths, starting at around 680 nm at nearly every stage in the milk coagulation.

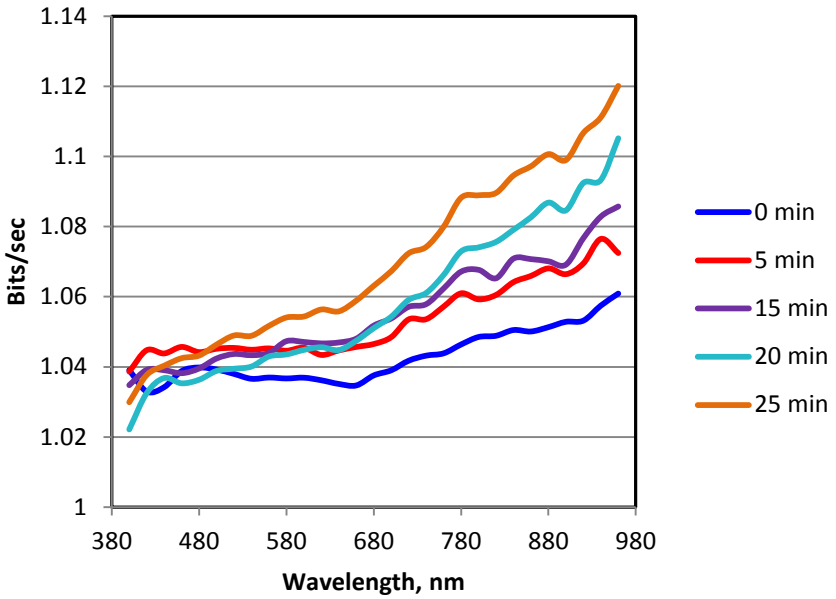


Figure 4.1.1 Backscatter signal over a broad spectrum taken at 5 minute intervals during coagulation of milk.

The method of analysis used for this test consisted of taking a ratio of two wave bands so that any interference with the signal would be negated by the fact that both wave bands would have experienced it. Based on the spectral responses one waveband was chosen from the infrared spectrum (1000 nm) and the other chosen from the visual light spectrum (500 nm), as both of these bands showed a response that was made clearer when placed in ratio to one another. The coagulation curve in *Figure 4.1.2* shows the resulting ratio which appears similar to a typical coagulation curve.

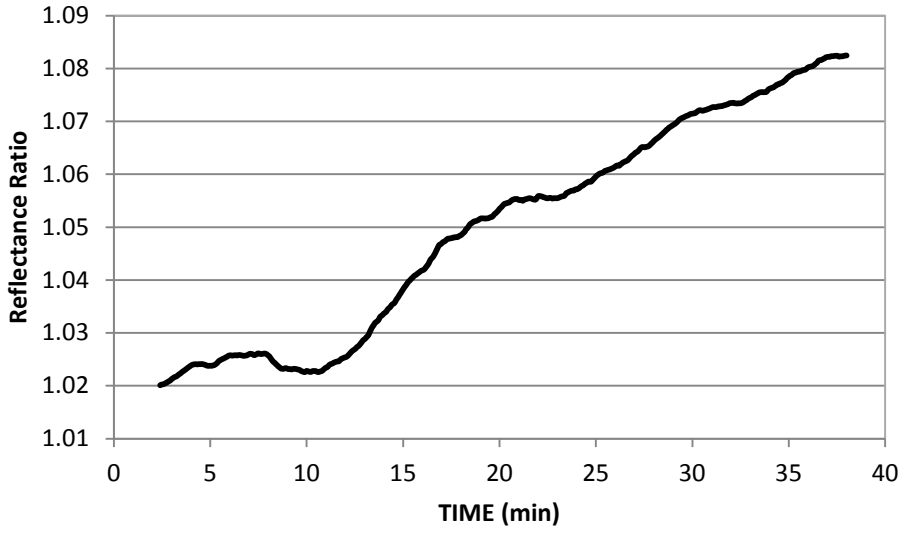


Figure 4.1.2. Reflectance ratio obtained by dividing the light scatter response for the infrared wave band (1000 nm) by a visible waveband (500 nm).

The coagulation curve seen in *Figure 4.1.2* was considered sufficient to validate the proof of concept that a response similar to a typical milk coagulation curve was obtainable from a non-contact light scatter sensor.

The second part of the objective for this test was to determine the wavelengths that provided the better responses to milk coagulation. *Figure 4.1.3* shows the decimal change in the light backscatter signal during the coagulation process. It is noted that very little change in reflectance from coagulating milk occurred below 600 nm and a substantial change occurred above 600 nm with the maximum change occurring around 980 nm.

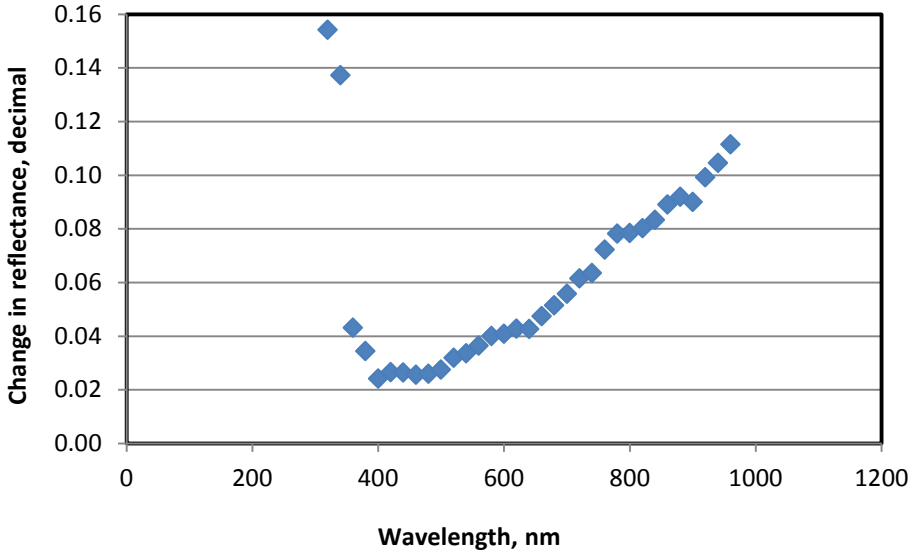


Figure 4.1.3. Changes in light scatter intensities as a function of wavelengths during the coagulation of milk. Note: there was little change in intensity for wavelengths below 600 nm.

It was originally theorized after the initial analysis of the data that a signal consisting of the wavelengths below 600 nm would provide a steady reference when compared with the infrared signals higher than about 700 nm. Thus a ratio of the infrared to visible light spectra should show a discernible "coagulation profile". *Figure 4.1.4* shows the ratio of diffuse reflected infrared light (700 to 900 nm) to visible light (400 to 600 nm).

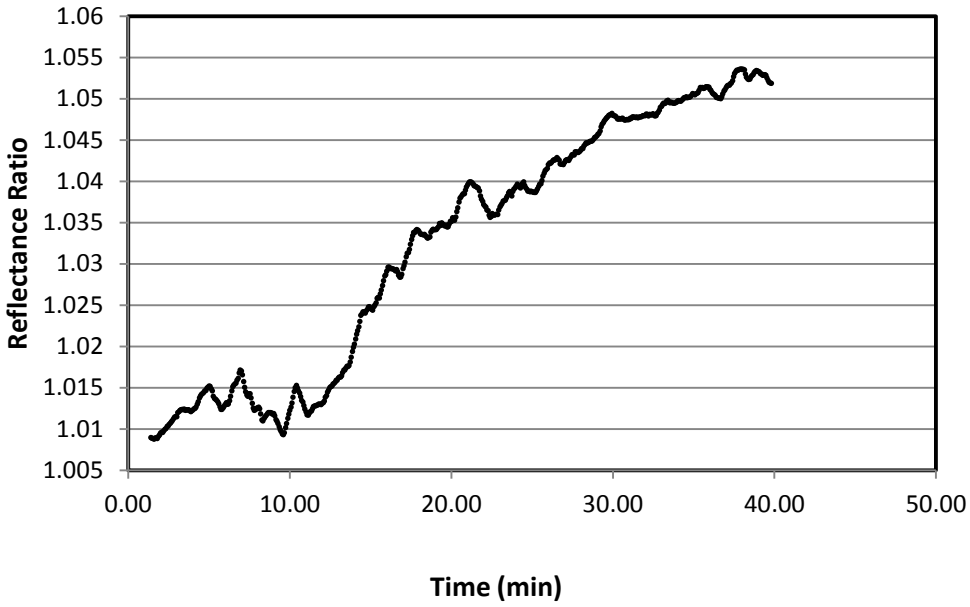


Figure 4.1.4 Ratio of light scattered intensities averaged over the waveband from 700 to 900 nm to that for the waveband from 400 to 600 nm.

Likewise, a coagulation response was generated by taking the ratio of two infrared wavelengths. *Figure 4.1.5* shows a ratio of the light scattered intensities for the infrared wavelengths 920 nm and 820 nm.

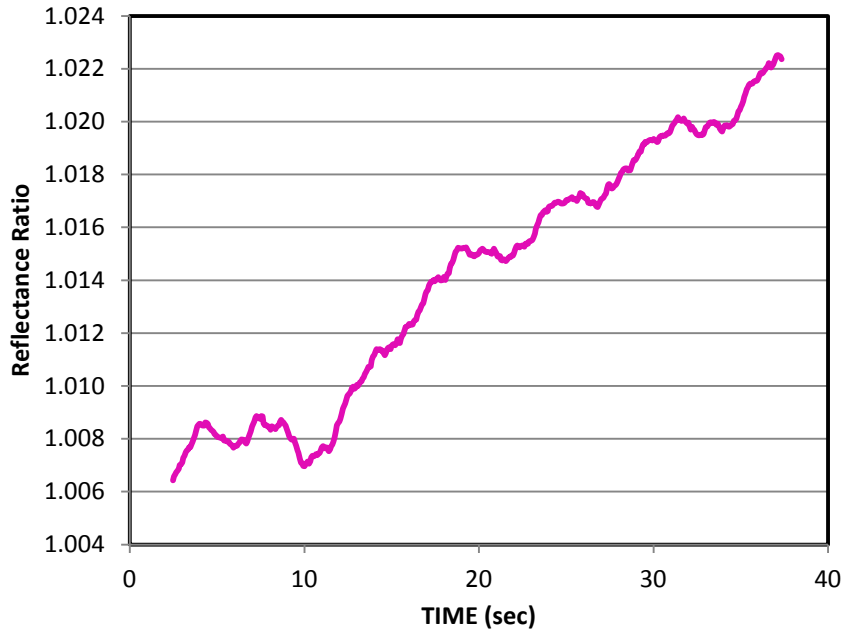


Figure 4.1.5. Ratio of the light scattered intensities for the infrared wavelengths 920 and 820 nm.

As the coagulation profile for the two infrared wavelengths was very similar to the profile from the ratio of the infrared wavelengths to visible wavelengths, it was decided that in the next experiment, two infrared wavelengths should be used. This was a matter of convenience: as the two methods showed roughly the same results, dealing with only one section of the light spectrum would allow for the use of a more sensitive light detector in further experiments.

4.2 Experiment 2 – Comparing Two infrared LEDs

The light backscatter response for two infrared LEDs (880 and 970 nm) was tested with a TSL257 visual light detector (400 to 1100 nm) and TSL 267 infrared detector (800 to 1100 nm). Data collected by Detector 1, TSL257, for coagulating skim milk are shown in *Figure 4.2.1, Figure 4.2.2, and Figure 4.2.3. Figure 4.2.1* gives the reflectance ratio of each wavelength (880 and 970 nm) collected by the TSL 257 detector, and *Figure 4.2.2* shows the ratio of the two wavelengths.

Figure 4.2.3 gives the reflectance of the ambient light off the surface of the milk, which was taken when the LEDs were in the OFF position.

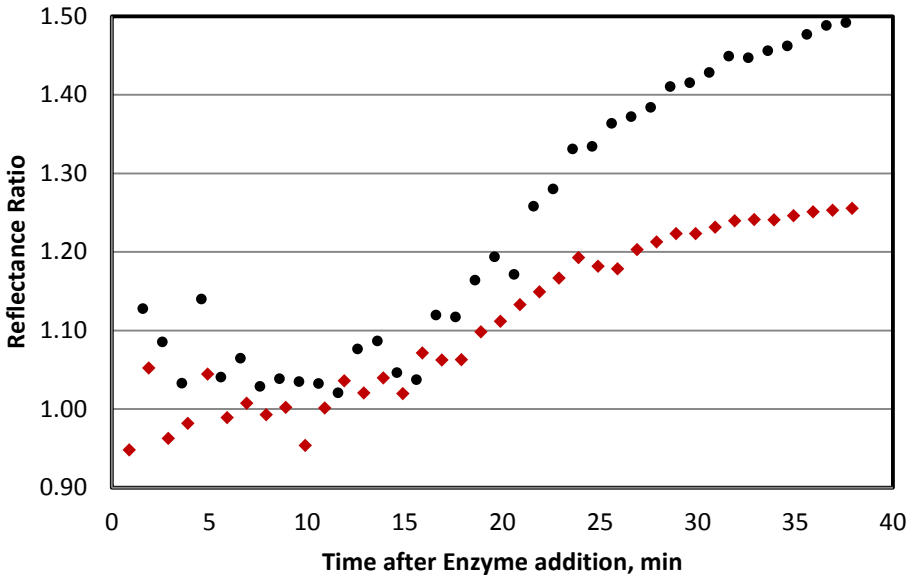


Figure 4.2.1. Reflectance ratio of 880 and 970 nm wavelengths in skim milk collected by the TSL257 visible detector.

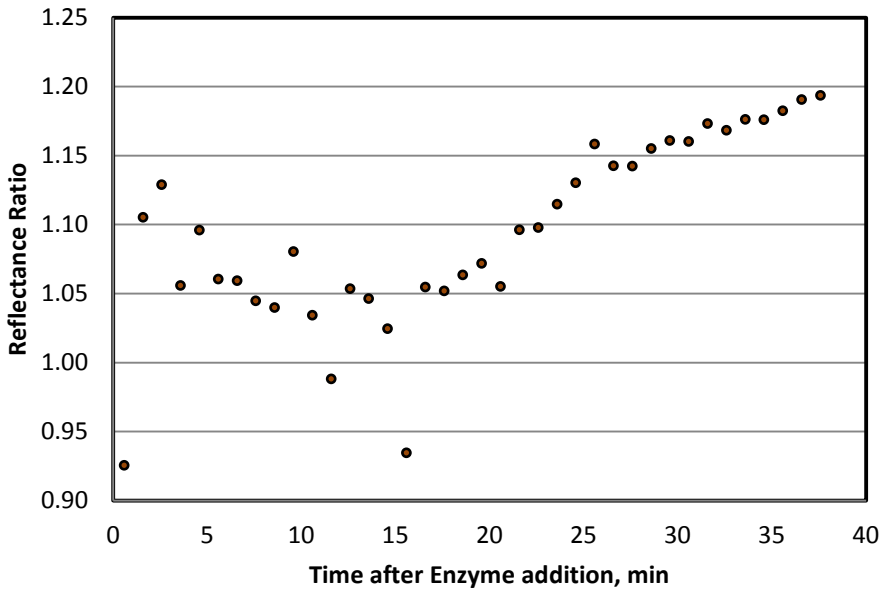


Figure 4.2.2. Ratio of light scatter intensities for 970 nm/880 nm for coagulating skim milk collected by the TSL257 visible detector.

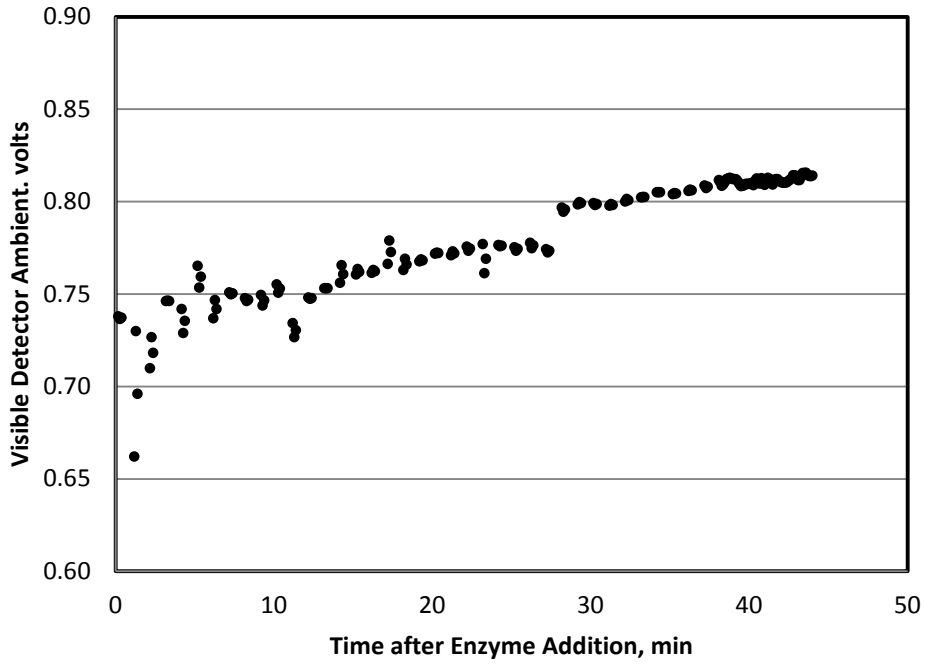


Figure 4.2.3. Ambient light intensity scattered measured by the TSL257 visible detector from the surface of coagulating skim milk.

The graph of output from Detector 1 in *Figure 4.2.1* shows the "coagulation profile" for both the 880 nm and 970 nm LEDs. The response was greater for the 970 nm (50% increase) as compared to the 880 nm (25% increase). The ratio of the two wavelengths (970 nm/880 nm) for Detector 1 in *Figure 4.2.2* displays a discernible coagulation profile, but had considerable noise at the beginning of the curve. This noise was partially a result of the method of data collection, in which the coagulation sample was prepared outside of the milk vat and poured into the vat after addition of the enzyme. The ratio of signals for Detector 1 had an increase of 20%. The ambient visible light (*Figure 4.2.3)* gave a signal of 0.75 volts or above, which was equal to or greater than the corrected signal response. This was a very significant signal, due possibly to movement around the instrument and possibly to increase in the room temperature and changes in the lighting of the room.

Data collected by Detector 2, TSL267, which detects infrared light, for skim milk is shown in *Figure 4.2.4, Figure 4.2.5, and Figure 4.2.6. Figure 4.2.4* shows the reflectance ratio for both wavelengths collected by the infrared detector, and *Figure 4.2.5* shows the ratio of the two wavelengths together. *Figure 4.2.6* gives the reflectance of the ambient light off the surface of the milk, which was taken when the LEDs were in the OFF position.

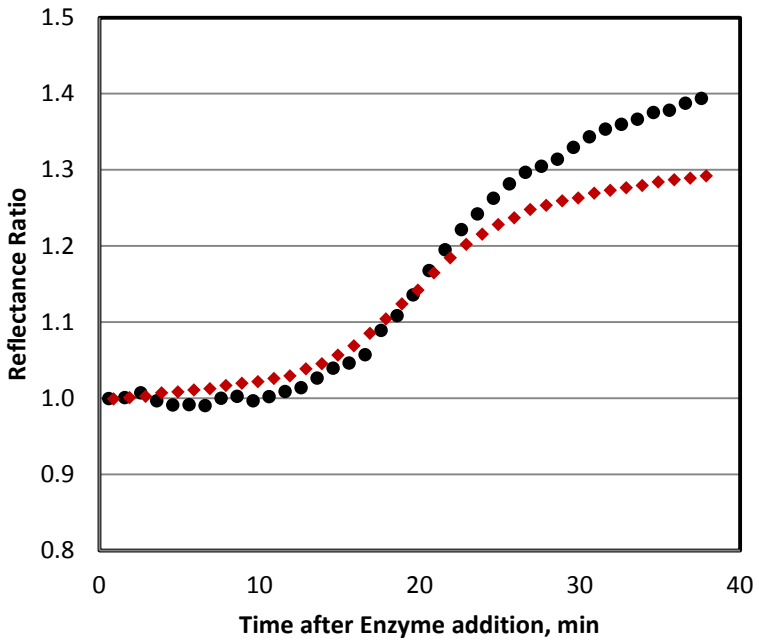


Figure 4.2.4. Reflectance ratio of coagulating skim milk using a 970 nm (black symbols) and 880 nm (red symbols) LEDs. The response was measured with an infrared detector, TSL267. Both signals were corrected for ambient light.

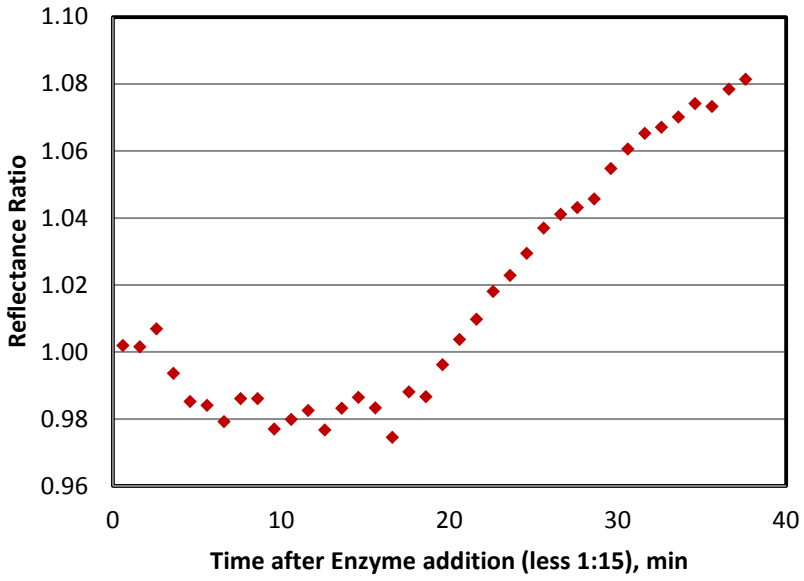


Figure 4.2.5. Reflectance ratio (970 nm/880 nm) of coagulating skim milk using a 970 nm and 880 nm LED. The response was measured with an infrared detector, TSL267.

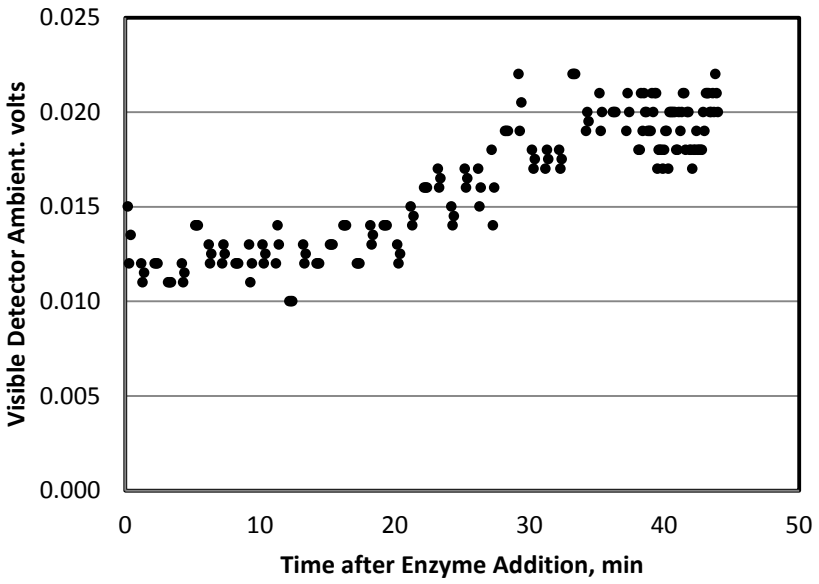


Figure 4.2.6. Ambient light scatter measured by the infrared detector, TSL267, during the coagulation of skim milk.

The coagulation profile for both wavelengths shown in *Figure 4.2.4* was very smooth with a 30% increase for the 880 nm light and a 40% increase for 970 nm. This smooth signal was most likely due to the fact that there was very little ambient infrared light (*Figure 4.2.6)* and therefore movement about the room or changes in the room's lighting would have little to no effect on the signal. The ratio as shown in *Figure 4.2.5* shows the characteristic coagulation curve but with noise.

When the test was repeated with two percent milk, similar results were observed, as can be seen in *Figure 4.2.7, Figure 4.2.8,* and *Figure 4.2.9*. The signal responses were decreased because of the effect of fat diminishing the change in response and consequently the signals displayed more noise.

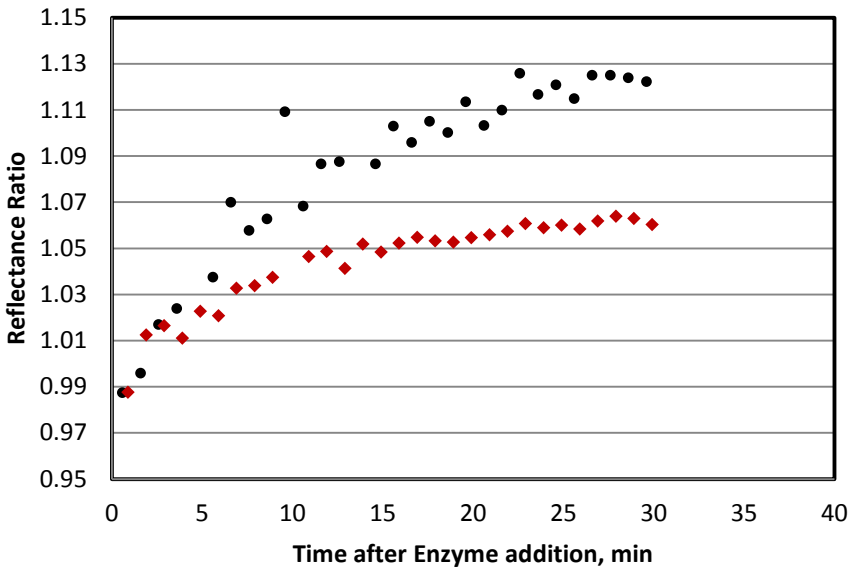


Figure 4.2.7. Reflectance ratio of 880 nm (red symbols) and 970nm (black symbols) wavelengths for two percent milk collected by the TSL257 visible detector.

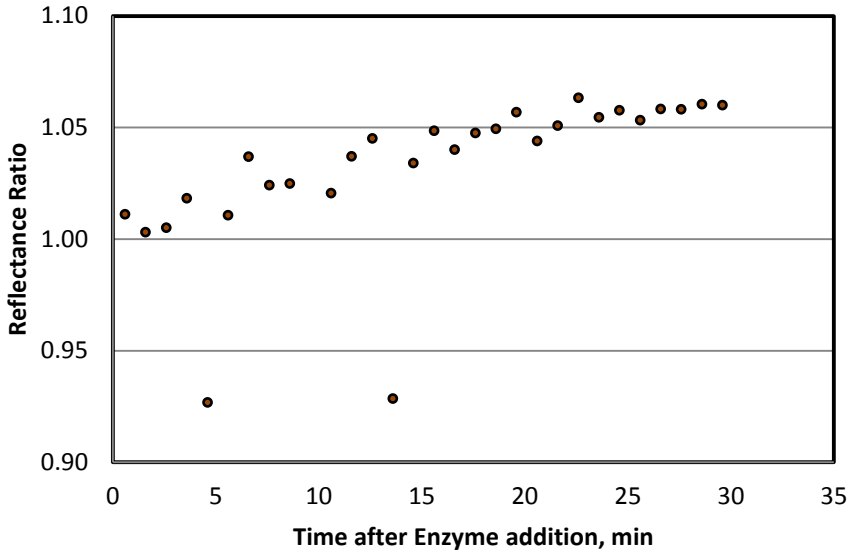


Figure 4.2.8. Reflectance ratio of 970 nm/880 nm in two percent milk collected by the TSL257 visible detector.

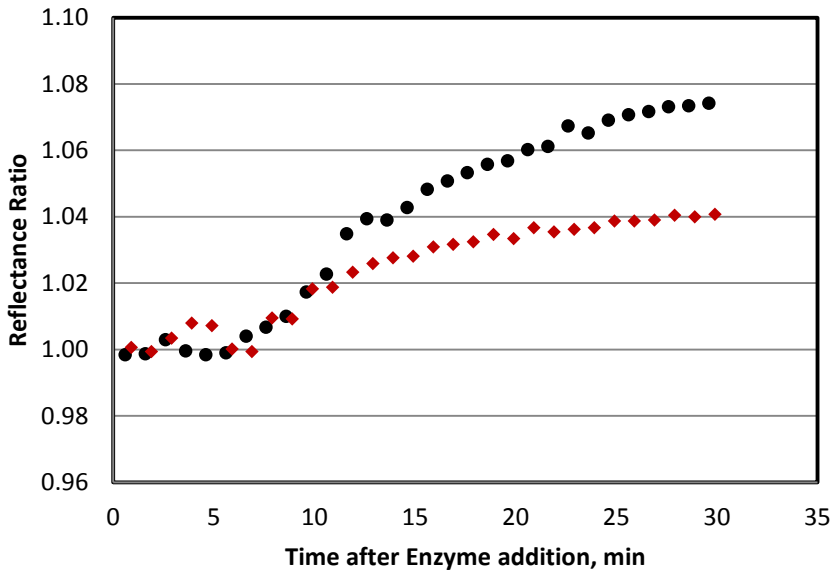


Figure 4.2.9. Reflectance ratio of 970 nm (black symbols) and 880 nm (red symbols) in two percent milk collected by the TSL267 infrared detector.

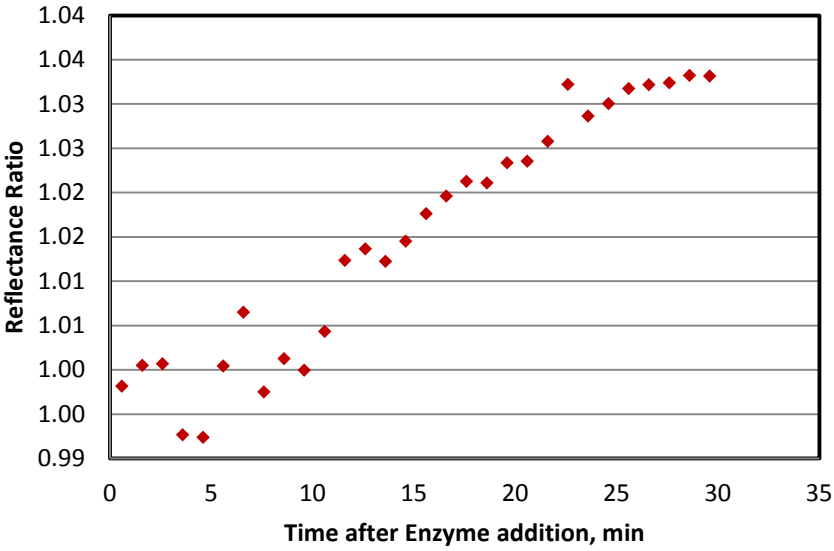


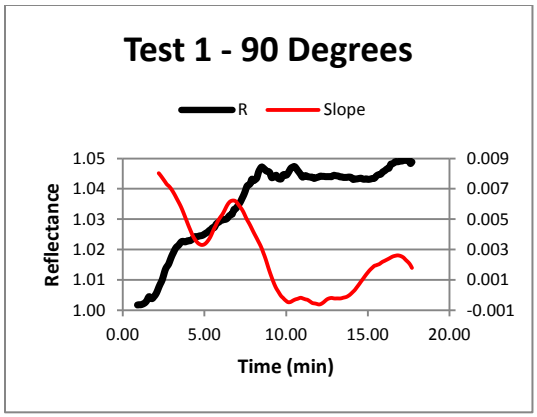
Figure 4.2.10 Reflectance ratio of 970 nm/880 nm in two percent milk collected by the TSL267 Infrared Detector.

For the two tests, it was clear that the largest response was detected with the 970 nm LED. There was a 48% signal increase with skim milk and 10% signal increase with two percent

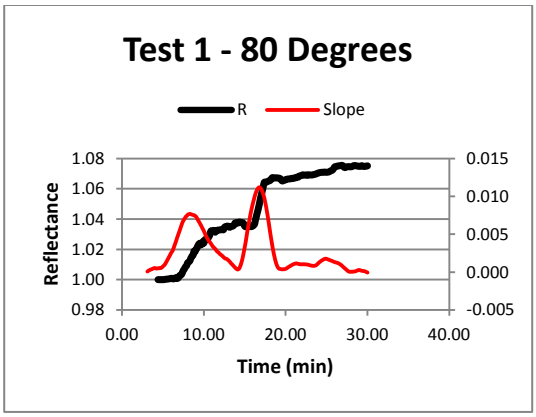
fat milk. The response for the 880 nm LED had a 27% increase for skim and a 5% increase for two percent fat milk. Thus the response for 970 nm LED was approximately double the response for 880 nm LED. The infrared detector showed less of a signal increase in both tests than the visible detector, though the infrared gave a much smoother signal. The higher signal for the visible detector likely resulted because of its high spectral responsivity to the 880 nm LED. As shown in *Figure 3.3.3.2*, the peak of the responsivity of the TSL257 light detector is around 700 nm. However, its responsivity does not wane significantly until roughly 900 nm, and doesn't dissipate completely until the 1100 nm range. Therefore, an 880 nm wavelength would fall well within the region in which the TSL257 is most responsive, and the 970 nm LED would produce a response as well. The TSL267 infrared detector, however, does not have a high responsivity at 880 nm. In fact, most of the data collected by the TSL267 was from the 970 nm LED. Therefore, the higher signal for the visible detector resulted because it had a broader spectrum of responsivity, and could therefore collect a substantial amount of information from both LEDs, while the infrared detector gathered most of its information from only the 970 nm LED.

4.3 Experiment #3 – Angle Determination

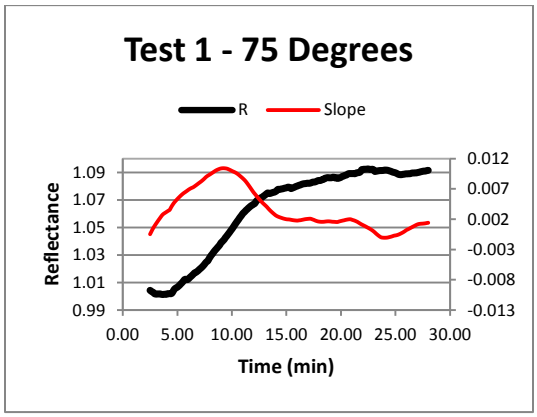
The light backscatter response for infrared LEDs (970 nm) collected by the TSL267 infrared detector (800 nm to 1100 nm) for the coagulating raw milk samples at the various angles tested is shown in *Figure 4.3.1 (A)-(E)* for replication 1*, Figure 4.3.2 (A)-(E)* for replication 2, and *Figure 4.3.3 (A)-(E)* for replication 3.



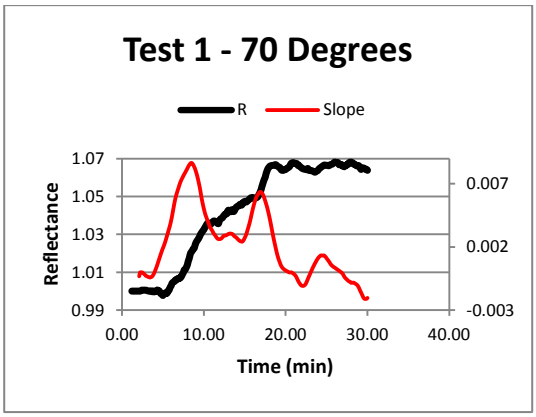
(A)



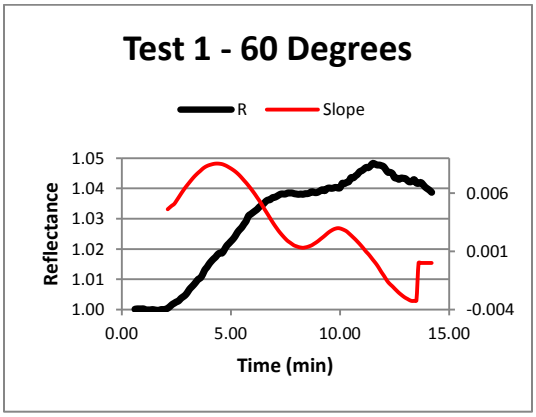
(B)



(C)

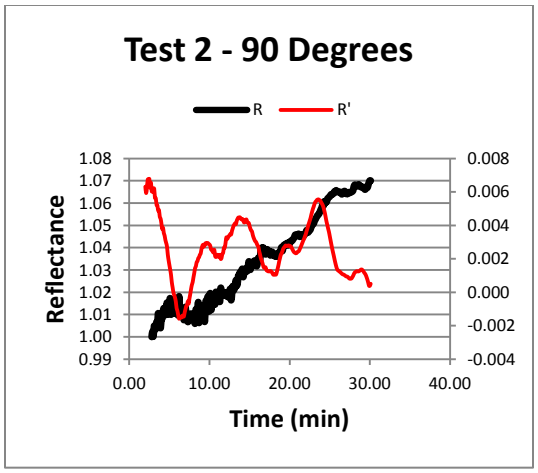


(D)

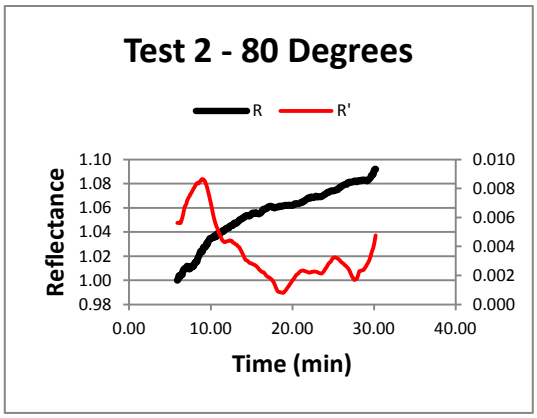


(E)

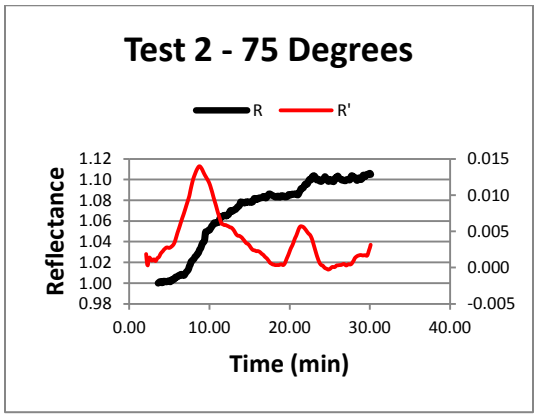
Figure 4.3.1. Reflectance ratios and slope of coagulation curve at each angle tested during Test 1.



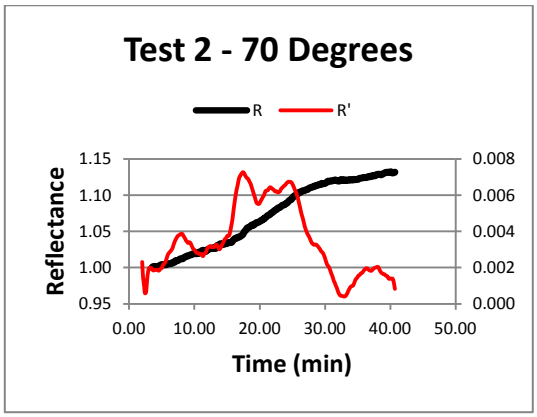
(A)



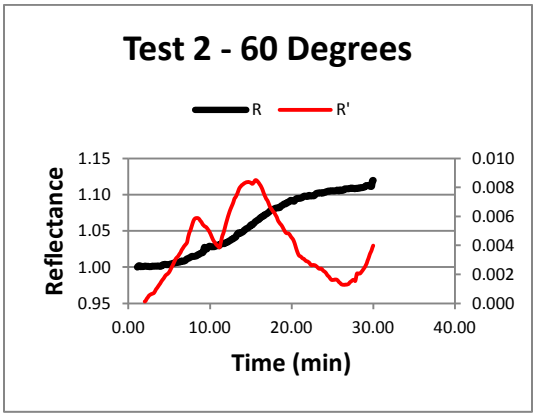
(B)



(C)

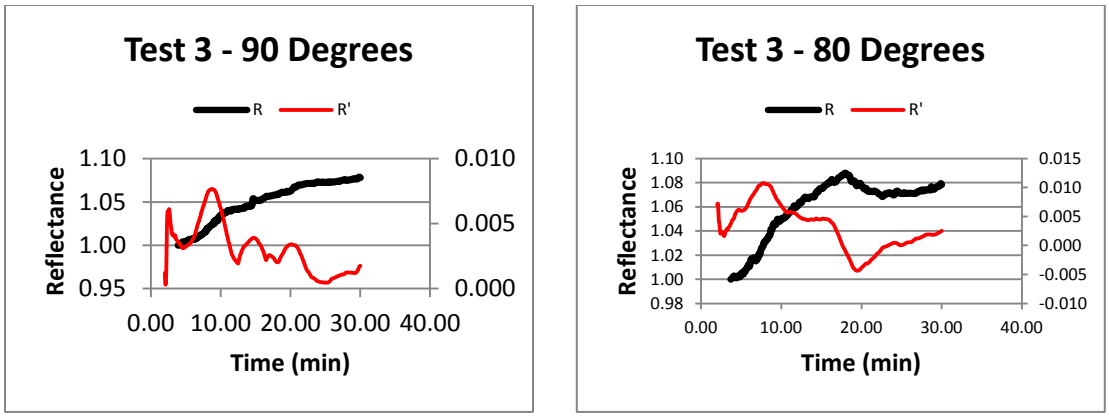


(D)



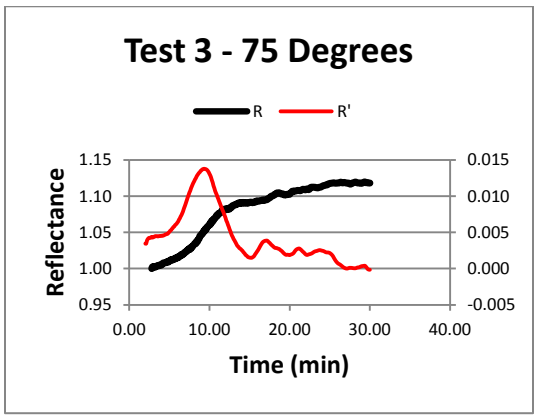
(E)

Figure 4.3.2. Reflectance ratios and slope of coagulation curve at each angle tested during Test 2.

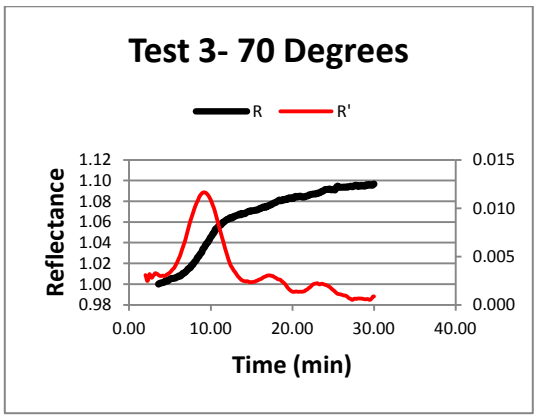


(A)

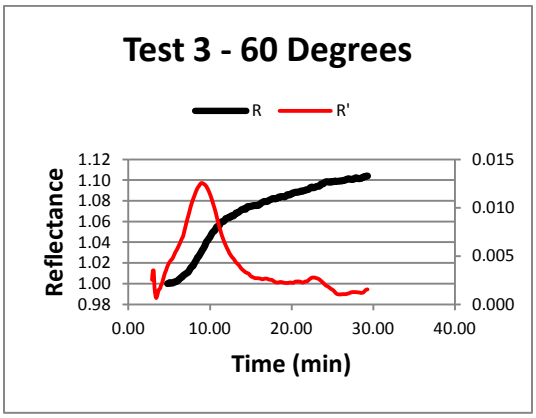
(B)



(C)



(D)



(E)

Figure 4.3.3. Reflectance ratios and slope of coagulation curve at each angle tested during Test 3.

The graphs in the figures above show the coagulation profiles for three repetitions of each angle tested. In the first test, the coagulation profile with the detector and light source placed at 75° above the milk (*Figure 4.3.1 (C))* showed the greatest response (9% increase). Positioning the detector and light source at 60° (*Figure 4.3.1 (E))* showed the least response, with only a 4% increase in signal strength. The smoothest signal was also seen at 75° in this test, though both 70° (*Figure 4.3.1 (D))* and 60° showed discernible coagulation curves as well. However, positioning the detector and light source at 80° (*Figure 4.3.1 (B))* and 90° (*Figure 4.3.1(A))* produced noisy signals that were not easily interpreted. In this test, when the light source and detector were positioned at both 60° and 75°, t_{max} was very apparent. When positioned at 80° or 90°, however, it was difficult to define a clear t_{max}.

When the test was repeated, the coagulation profile with the detector and light source placed at 70° above the milk (*Figure 4.3.2 (D))* showed the greatest response (12% increase). However, 60° (*Figure 4.3.2 (E))* gave a good response, with an 11% signal increase, and the signal for both 75° (*Figure 4.3.2 (C))* and 80° (*Figure 4.3.2 (B))* increased by 9%. The smoothest signals were seen when the detector and light source were placed at 60° above the milk or 70° above the milk, though t_{max} was most easily discernible for 80°, 75°, and 60°. The signal for the 90° position (*Figure 4.3.2 (A))* in this test was extremely noisy, and therefore little usable information was collected from that signal.

In the second repetition of the test, the coagulation profiles with the detector at 75°, 70°, and 60°, respectively, showed the greatest increase in signal strength. Though 75° (*Figure 4.3.3 (C))* gave the best response (12% increase), the signal for both 70° (*Figure 4.3.3 (D))* and 60° (*Figure 4.3.3 (E))* increased 10%. When the detector and light source were placed at 75°, 70°, and 60° in this test, very smooth coagulation profiles were observed, and t_{max} was easily discernible. It should be noted also that t_{max} was easily found in the coagulation profiles for 90°

(*Figure 4.3.3 (A))* and 80° (*Figure 4.3.3 (B))*, though the signals for those angles were noisier and showed less of an increase (7%) than the other three signals.

It is clear from the information above that there was a substantial amount of variability in the tests performed. There are many potential reasons for this variability, including changes in ambient temperature in the lab, human error, and changes in the milk used for testing (the fat particles in unhomogenized whole milk are not uniformly sized, milk was tested at different times, etc.)

A statistical analysis was performed on R_{max}, the value of the reflectance ratio at t_{max}, for each angle tested. *Table 4.3.1* gives the values of R_{max} for each angle.

Table 4.3.1 Rmax values for each angle in each repetition of the test.

Angle	Rmax Rep 1	Rmax Rep 2	Rmax Rep 3
90	1.032	1.029	1.024
80	1.035	1.023	1.027
75	1.039	1.032	1.052
70	1.024	1.046	1.035
60	1.018	1.063	1.032

The following ANOVA table (*Table 4.3.2)* shows that the angle at which the sensor and light source are mounted above the milk is significant.

Table 4.3.2 ANOVA table for Rmax vs Angle

Source	DF	Type I SS	Mean Square	F Value	P-value
ANGLE	4	0.00057807	0.00014452	8.21	0.0328

The P value shown in *Table 4.3.2* is 0.0328, which means that it can be said with at least 95% confidence that the means of R_{max} at each angle tested are not statistically equal.

An interaction plot for R_{max} is shown in *Figure 4.3.4*. This plot shows the R_{max} values for each angle, when the effects of differences in signal strength are taken into account.

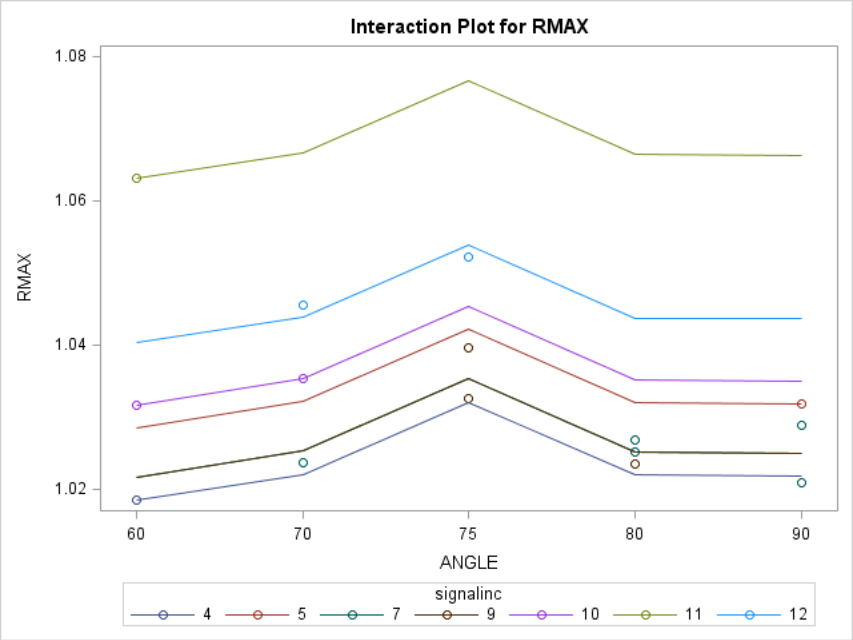


Figure 4.3.4 Interaction plot for R_{max} showing the effect of each angle on R_{max} when the influence of signal strength is negated.

It is evident from the plot in *Figure 4.3.4* that an optical system mounted above the vat at a 75° angle consistently receives the best coagulation signal at t_{max}. As the determination of t_{max} is one of the most essential reasons for monitoring light backscatter data during coagulation, it is clear that a 75° angle would be the best angle at which to mount a sensor and light source above a milk vat.

4.4 Experiment #4 – Angle Verification

The change in light scatter intensity in the light backscatter response for infrared LEDs (970 nm) collected by the TSL267 infrared detector (800 nm to 1100 nm) for the scans made at various angles of coagulated and uncoagulated skim and unhomogenized milk samples are shown in *Figure 4.4.1* and *Figure 4.4.2.*

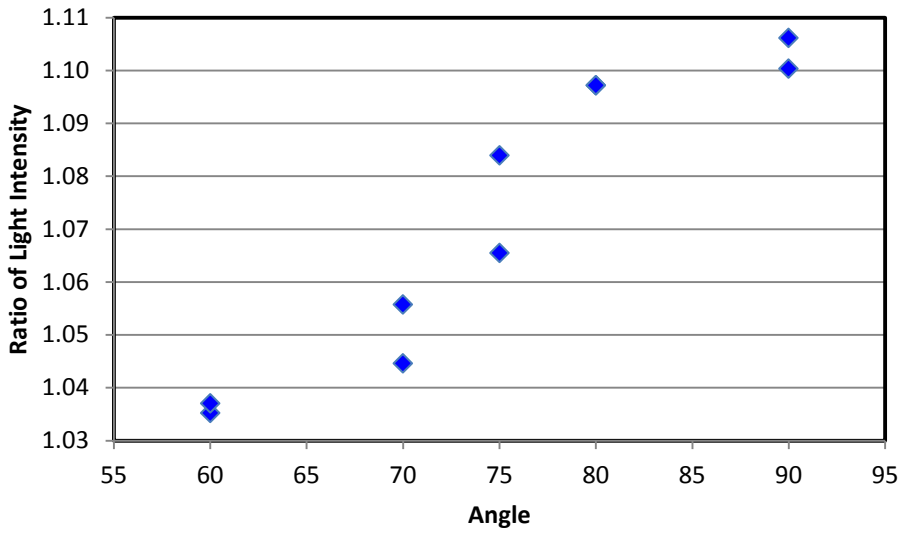


Figure 4.4.1./ Ratio of light intensity for coagulated vs. uncoagulated unhomogenized whole milk taken at various angles.

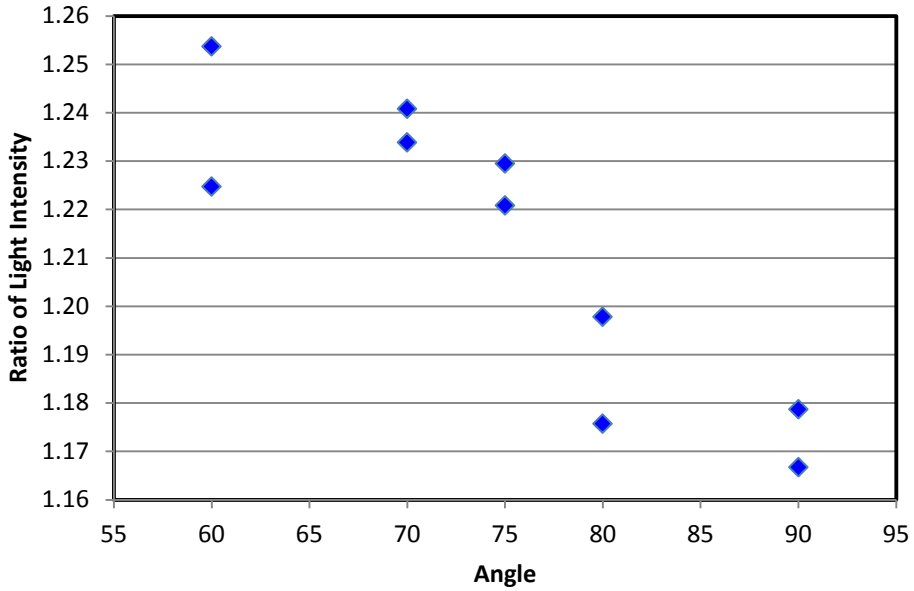


Figure 4.4.2. Ratio of light intensity for coagulated vs. uncoagulated skim milk taken at various angles.

The graphs shown in *Figure 4.4.1* and *Figure 4.4.2* are almost mirror images. The ratio of light intensities for coagulated vs. uncoagulated milk for unhomogenized milk has a positive correlation to the angle at which the scan was taken, while the ratio of light intensities for

coagulated vs. uncoagulated milk for skim milk has a negative correlation. The opposite effect of these two graphs is due to the fact that they represent *ratios* of light intensities, rather than the light intensities themselves. The unhomogenized milk has a positive correlation due to the fact that there are fat particles in the sample. During testing these fat particles may have interfered with the signal, giving the graph for the unhomogenized milk an entirely different shape than the graph for the skim milk.

Due to the variation in the data gathered during experimentation, statistical analysis was done on the data in *Figures 4.4.1 and 4.4.2* in order to determine if the findings in Experiment 3 were valid. The following tables, *Table 4.2.1* and *Table 4.2.2,* show that after accounting for the distance at which each scan was taken, the slope of the graph of the angles at which the scans were taken is statistically significant.

Table 4.2.1 Results of t-test on scans of ratio of coagulated to uncoagulated skim milk.

Parameter Estimates					
Variable	DF	Parameter Estimate	Standard Error	t Value	P-value
Intercept	1	1.40128	0.06011	23.31	<.0001
angle	1	-0.00252	0.00079443	-3.17	0.0132

Table 4.2.2 Results of t-test on scans of ratio of coagulated to uncoagulated unhomogenized whole milk.

Parameter Estimates					
Variable	DF	Parameter Estimate	Standard Error	t Value	P-value
Intercept	1	0.93112	0.03462	26.90	<.0001
angle	1	0.00192	0.00045756	4.21	0.0030

The P value shown in *Table 4.2.1* is 0.0132, and the P value in *Table 4.2.2* is 0.0030, which means that it can be said with at least 95% confidence that the slope of the ratio of light intensities at each angle tested are not statistically equal. This confirms the results of Experiment 3, that the angle at which the light source and sensor are placed above the vat has an effect on the backscatter signal that can be collected off of the surface of milk coagulating in the vat.

CHAPTER 5: CONCLUSIONS

5.1 Experiment #1 – Evaluation of Sensor Concept

The data from this experiment shows that a coagulation curve can be obtained from measurement of light scatter using a light source and backscatter detector positioned above the surface of the milk, rather than with a sensor in direct contact with the milk. The results also showed that not only can light scatter be detected from visible light, but that it can be detected in the infrared wavelengths as well. Additionally, the change in the scatter intensity is greater in the infrared region. The change in light scatter in the visible region between 400 nm and 600 nm is relatively small. This region could potentially act as a base reference to be used in conjunction with an infrared signal for establishing a coagulation curve. Furthermore, simply taking a ratio of two infrared wavelengths produces a similar coagulation curve. Therefore, due to the fact that focusing on one section of the light spectrum allows for the use of a more sensitive light detector, infrared signals should be used in further testing of backscatter data collection above the surface of the milk. More specifically, wavelengths in the range of 800 to 1000 nm showed particular promise for establishing a coagulation curve.

5.2 Experiment #2 – Determination of Ideal Wavelength

The data from this experiment showed that an easily discernible coagulation curve was obtained using a 970 nm or an 880 nm LED and an optical sensor placed above the coagulating milk. The light scatter increase for the 970 nm LED was significantly greater and smoother than that for the 880 nm LED. The ratio of light scatter intensities for 970 nm/880 nm gives a coagulation curve that shows a signal increase of 20% for skim milk and 6% for two percent milk. Based on the above results, the wavelength that showed the most potential for use in non-contact detection and monitoring of milk coagulation was 970 nm.

The most effective detector for use in this application was the TSL267 High Sensitivity Infrared Light to Voltage Converter. Though this detector showed less of a signal increase than

the TSL257 visible detector in both the skim and two percent tests, it output a much smoother signal in both cases. This smoother signal likely resulted because the inherent infrared filter eliminated nearly all ambient light and the spectral responsivity of the TSL267 is very narrow. As eliminating ambient light is an effective means of getting a smooth signal, and covering a sensor that is being used in an actual cheese plant is impractical, using a detector that primarily detects light at very specific wavelengths is a viable option for reducing the effect of ambient light on the signal.

5.3 Experiment #3 – Angle Determination

The data from this experiment contained a substantial amount of variability. While the raw data seemed to show that mounting a sensor and light detector between 60° and 75° above a milk vat would produce the best backscatter signal for monitoring coagulation and predicting cutting time, a statistical analysis was necessary in order to verify these assumptions.

The statistical analysis showed that the angle at which the light source and sensor were mounted above a milk vat were significant for producing a usable backscatter signal. Furthermore, when the influence of signal strength was eliminated, it was apparent that the best angle at which an optical sensor and light source should be mounted above a milk vat to monitor coagulation and accurately predict cutting time was 75°.

5.4 Experiment #4 – Angle Verification

This experiment was performed to verify the results of Experiment 3. The data gathered during experimentation served to prove that the angle at which a sensor and light source were mounted above a milk vat was statistically significant for both skim and unhomogenized whole milk, both before and after coagulation.

5.5 Future Work and Recommendations

The next step in development of this technique for monitoring milk coagulation and predicting cutting time would be designing and constructing a non-contact optical sensor that could function effectively in a commercial cheese-processing facility. The frame apparatus used to mount the optical components at desired angles should be adapted to fit a commercial cheese vat. Though as the target market for this design are artisan cheese makers who don't have standardized vats, the frame structure should be relatively flexible and adaptable to different vats. The simple LED and detector configuration should be adapted to a more standardized design and encased in a more stable structure that can be mounted easily to the new frame structure.

5.6 *Potential Impact*

The main objective of this study was to develop a scientifically accurate method for coagulation monitoring and cutting time prediction in cheese making that could be implemented by smaller cheese producers and artisan cheese makers. This study proved that an optical sensor could be mounted above a cheese vat that can accurately monitor coagulation and predict cutting time. This sensor technology could allow these producers to meet the quality standards set by larger manufacturers without the cost involved in installing a more expensive sensor.

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