IMPROVED CONTROL OF CHEESE MANUFACTURE THROUGH CONTINUOUS VAT MONITORING OF COAGULATION PARAMETERS USING THE HOT WIRE METHOD

by

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ABSTRACT

Improved Control Of Cheese Manufacture Through Continuous Vat Monitoring Of Coagulation Parameters Using The Hot Wire Method

by

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The hot wire method, with pH and temperature sensors, was evaluated to determine its usefulness and application for cheese production automation. Coagulation of milk substrate was measured with the hot wire instrument and by four other methods: Formagraph, Brookfield® viscometer, Omnispec™ bioactivity monitor, and Sommer and Matsen rolling bottle method. The hot wire, using the time at maximum slope, detected coagulation before methods that measure resistance to shear, and after methods that measure light reflectance. Coagulation time was not significantly different from the industry standard rolling bottle method used by Sommer and Matsen. The hot wire instrument was also used to distinguish samples that formed curd at different rates. This was accomplished by measuring the rate of temperature change of the hot wire probe during curd formation. Milk samples of varying protein, fat, and calcium concentrations were prepared to determine if the instrument could be used to predict a consistent curd cut-point. The pH level was also adjusted, and rennet additions were varied. Coagulation was monitored simultaneously with the hot wire system and a Formagraph. All five factors (pH, calcium, fat, protein, and rennet) had significant effects on cut time estimations (CT_{20}) on the Formagraph. Linear correlations (R^2) ranging from .74 to .94
were obtained using stepwise regressions when comparing hot wire and compositional data with the Formagraph.

A Formagraph was used to measure effects of calcium, pH, and rennet changes on the coagulation properties of late lactation milk. Calcium, pH, and rennet treatments significantly affected the coagulation parameters measured by the Formagraph. However, response among the poor coagulating samples to treatments to improve coagulation was sample dependent. General composition and SDS-PAGE fractionation data could not be used as an indicator of poor or good coagulability of samples.

The hot wire method worked well for monitoring coagulation time and curd firming rate, but did not measure maximum curd firmness well. Curd firming rates determined from the hot wire data are acceptable for estimation of a curd cut time. Added benefits of the hot wire method for monitoring cheese manufacture are that stirring, coagulation, and healing of curd can also be measured. Therefore, the rates of change of important parameters, such as pH, temperature, and coagulation during critical processing steps, can easily be determined by a computer and displayed, printed out, or saved for future evaluation.
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Michael J. LeFevre
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# LIST OF ABBREVIATIONS AND SYMBOLS

- **1.8X** = 1.8 times (x) concentrated by volume reduction
- **12 BIT resolution** = Voltage range\(/2^{12}\)
- **AC** = Alternating current
- **AD** = Analog-to-digital converter
- **BIT** = One binary digit, Either 0 or 1
- **C** = Celsius
- **CT\(_{20}\)** = Cut time determined by the Formagraph
- **CT'** = Coagulation time measured by the HWP at the time of \(\Delta T'\) max.
- **CT''** = Coagulation time measured by the HWP at the time of the maximum value of \(\Delta T''\).
- **CT'\(_0\)** = Coagulation time measured by the Omnispec\textsuperscript{TM}
- **CT\(_f\)** = Coagulation time measured by the Formagraph
- **CT\(_{sm}\)** = Coagulation time measured by the Sommer and Matsen method
- **CT\(_v\)** = Coagulation time measured by the Brookfield\textsuperscript{®} viscometer
- **DAC** = Digital-to-analog converter
- **DC** = Direct current
- **DIM** = Days in milk production
- **DLL** = Dynamic link library
- **\(\Delta T\)** = Difference in temperature between the surface of the HWP and \(T_S\)
- **\(\Delta T'\)** = Rate of change of \(\Delta T\) (°C/s)
- **\(\Delta T'\) max** = Maximum rate of change of \(\Delta T\) during coagulation
- **\(\Delta T''\)** = Rate of change of \(\Delta T'\) (°C/s\(^2\))
- **GLD** = Glucono-\(\delta\)-lactone
h = Hour
HWP = Hot wire probe
min = Minutes
MSE = Mean square error
P&E = Power and event station
PC = Personal computer
PROC GLM = General linear models procedure on SAS
PROC REG = Regression procedure of SAS
RU/ml = Rennet units per ml of milk
RTD = Resistance temperature detector
s = Second
SAS = Statistical Analysis System™
t = Time
T_s = Ambient sample temperature
v = Volume
wk = Week
wt = Weight
X = Times
GENERAL INTRODUCTION

Many instruments have been invented to measure milk coagulation during cheese manufacture. Moving parts, calibration difficulty, removal during cutting, lack of sensitivity, and other problems have prevented cheese makers from selecting an instrument to improve control of curd cutting during cheese manufacture. This project was initiated after information regarding a new coagulation measuring instrument was invented. With no moving parts and computer connection, the hot wire probe instrument promised to finally provide a means for fine-tuning the cutting of cheese curd. The general objective of the project was to validate the ability of the instrument to measure coagulation by comparing its data to that of other instruments. Coagulating milk, with pH differences, calcium addition and varied rennet levels could then be measured with the instrument. Similar data could then be obtained from milk that coagulates poorly, such as late lactation milk. Such data could be used to program a system to help manufacturers to predict optimum timing of curd cutting, along with appropriate levels of rennet and calcium addition.
LITERATURE REVIEW

Cheese manufacture is a complex process in which casein, fat, and colloidal salts are concentrated from milk. Chemical, biochemical, and physical means are used to manufacture specific cheeses, even with unique flavor and texture profiles. Enzymes are used to destabilize casein micelles, which aggregate to form a fragile curd that strengthens over time. Once the curd is cut, whey is expelled and curd particles mat together unless agitated. During this process approximately 90% of the casein proteins and 93% of the fat globules are trapped in the curd (34). Meanwhile lactic culture organisms produce acid and flavor compounds characteristic of the specific cheese. Bacterial acid production is controlled by manipulation of time and temperature conditions and by dilution with water or removal of whey. Such adjustments to the whey concentration affect the availability of the carbohydrate used for acid production. The curd is stirred, heated, salted, packed, and aged to form the final product. The manufacturer selects conditions that produce cheese with the desired characteristics, but external factors such as raw milk quality and treatment of the milk may affect cheese production even though a make-procedure is followed correctly.

Microbial Quality

Microbial quality of raw milk must be maintained prior to cheese manufacture to produce a good quality cheese. Bacteria and somatic cells in raw milk degrade milk proteins and produce off flavors due to enzymic reactions. Cheese yield and coagulation are adversely affected by high somatic cell counts (2, 23, 24).

Acidity

The pH of milk at enzyme addition plays a major role in cheese manufacture. Both rennet clotting time and aggregation of micelles are pH dependent (4, 10, 21, 31).
Higher pH levels can cause decreased cheese manufacturing efficiency. As the pH level is increased above 6.6, the enzyme reaction rate and the micelle aggregation rate are reduced (19). This is especially true for porcine pepsin (5).

Control of acid production becomes more important once the curd is cut. While the curd is in the whey, bacterial acid production induces whey expulsion from the curd. Colloidal calcium phosphate dissociates and can leave the curd as the whey is expelled (5, 29). The amount of calcium and colloidal calcium phosphate that remain in the curd affect the final texture, meltability, and buffering capacity of the curd (5).

Suitability of Milk for Cheese Manufacture

Some milk is not suitable for cheese manufacture due to combinations of genetic variation and differences in casein composition (8, 11, 22). External factors such as diet of the cow (24) and season of the year (13, 32) can affect composition and enzymic coagulation of milk. Even milk with good coagulation properties can be treated in ways that reduce its effectiveness for cheese manufacture. For example, a weak curd may be formed after excessive heat treatment, homogenization, or cold storage of milk (2, 9).

During the course of lactation, coagulation properties of milk for cheese manufacture may also change (7, 8, 16, 21, 26). Milk that coagulates poorly is often obtained during late lactation (11, 22). Okigbo et al. (22) showed that the stage of lactation has a significant effect on the rennet-coagulation time, curd firmness of rennet-coagulated milk, and estimations of cheese yield.

Control of Cheese-Making

While the size and automation of cheese-making facilities vary considerably, control and timing of cheese manufacturing is necessary for consistent quality and yields. Characterized, single-strain, concentrated lactic cultures and standardized coagulants have helped manufacturers to schedule make-procedures to maximize use of available vats for
cheese manufacture. Because of the difficulty in monitoring curd formation and acid production in closed vats, many manufacturers set and cut the cheese curd based on time schedules (33). Injection ports are available for manual removal of sample for periodic titratable acidity and pH monitoring. Rapid identification of slow or dead vats is important in cases where many vats are operated simultaneously, since several vats for cheese manufacture may be inoculated with the same culture before the problem is detected. Errors associated with manual sampling and testing are avoided when continuous monitoring is available.

Continuous monitoring would be very useful for early assessment of coagulation or pH levels outside an acceptable range. Some cheese plants in Australia could save up to $100,000 per year (in reduced downgraded cheese and slow vats) when acid production is monitored continuously and steps are quickly taken to compensate for deviations from the norm (Peter Linklater, Sydney, Australia--personal communication at Logan, Utah--Oct, 1992). Occasionally, culture or coagulant addition is omitted accidentally by workers in plants where the ingredients are added manually (Peter Linklater, Sydney, Australia--personal communication at Logan, Utah--Oct, 1992). After such mistakes, the resultant milk is wasted and usually used for animal feed. Downgraded cheese also results from similar errors.

Aside from major errors in cheese manufacture that cause financial losses and quality reduction, subtle differences in the curd forming ability of milk can also lead to reduced yields and variation in cheese quality. Aleandri et al. (1) and Ng-Kwai-Hang et al. (20) evaluated cheese yields from milks with different coagulabilities. Decreased losses of milk fat, protein, and total solids were correlated with milk that clotted sooner, had a faster rate of firming, and was firmer 30 min after coagulation (18). Higher yields were obtained when milk with lower fat levels and faster firming rates were used for cheese manufacture (1). The highest yields were obtained when intermediate fat levels
and intermediate curd firmness were used for manufacture (1). In theory, if curd is cut before the coagulum is sufficiently firm, fat and protein may be lost into the whey (3). Conversely, if curd is cut when too firm, it could shatter, produce excessive fines, or impede syneresis (28).

Cutting curd at varied firmness has been investigated (3, 14, 15, 28). Bynum and Olson (3) increased yields by cutting curd at higher curd tensions (50-65% additional time from coagulant addition to cutting). Fat and casein levels in cheese increased without any change in moisture content. Riddle-Lawrence and Hicks (28) also reported increased dry matter yields when firm coagulum was cut (up to 130% of the normal set time). Increased fat was also observed when the firm curd was cut (28). Surprisingly, the highest yields and fat retention were obtained when curd was cut at the lowest curd tensions, which occurred at approximately 66% of the optimum or normal set time (28). However, cutting at low curd tensions was confounded with longer heal times. Mayes and Sutherland observed that a wider cut window was available without adverse effects (14). When economic effects were considered, increased yield was counterbalanced by excessive moisture retention when coagulum was cut between 116% to 200% of the normal cutting time. In further studies, Mayes and Sutherland (15) observed that a reduction of the setting time by as much as 40% had little effect on the moisture content or yield. They concluded that the use of curd firmness measurement was mostly useful in closed vat systems. If, for some reason, coagulation did not occur within the prescribed time, steps could be taken to correct the problem before further manufacture.

**Measurement of Coagulation**

Many instruments have been developed to monitor milk coagulation (6, 10, 12, 17, 18, 25, 27, 30). These instruments can allow the cheese manufacturer to cut the curd at uniform strength, and are especially useful in closed vats where the curd is not easily
evaluated for firmness. Inherently, a single optimum cutting point cannot be precisely determined for all cheese manufacturing because of the many factors that affect curd formation. But each manufacturer can use these tools to optimize the process and avoid expensive mistakes. A review of objective data from cheese production could also serve as a quality control aid since cheese is often evaluated months after initial manufacture.

Most instruments suitable for measurement in cheese vats dynamically measure a resistance or response to a shear force. Instruments that break the curd while measurements are taken, such as viscometers, are slowly moved or "creeped" through fresh curd to obtain correct data (12). Some instruments measure resistance to oscillation of a disc (27), sphere (25), or reed (17) suspended in the curd. These instruments must be sufficiently gentle to prevent shattering the curd that is being measured. Other instruments measure pressure waves or ultrasonic waves transmitted through the curd (13). Some of these instruments are difficult to calibrate and maintain and must be removed when the curd is cut.

More recently, instruments have been introduced that measure light reflectance (33), but this does not correlate with curd firmness (19). The development of a non-destructive, clean-in-place instrument acceptable to cheese manufacturers could improve the quality, yield, and consistency of cheese while fine-tuning the use of culture, enzyme coagulant, and CaCl₂ additions.

By monitoring temperature change around a heated probe, milk coagulation and cheese making can also be followed (10). The concept of the hot wire probe (HWP) viscosity monitor is fairly simple. A constant current is applied through a platinum wire within the probe. The change in voltage required to maintain the current is measured. This voltage varies as heat is removed from the probe by free or forced convection. The variation in voltage is thus related to the state of the milk around the probe (10).
References


OBJECTIVES

The objective of this research was to evaluate the hot wire method for monitoring and optimizing cheese manufacture from coagulant addition through curd cutting and heating. A computer-based system using temperature, hot wire, and pH sensors was developed to meet the research objective. The research objectives were:

1. To design and build a laboratory data acquisition and control system using a Zymate® II robot to provide sample repeatability in the collection of milk pH, temperature, and coagulation curve parameters.

2. To determine the ability of the hot wire system to detect differences in coagulation time and curd firmness, and to compare the hot wire instrument with other instruments commonly used to determine coagulation time.

3. To determine the portions of the hot wire curve that are most related to curd firmness and compare these data with Formagraph curd firmness values obtained during enzyme and acid coagulation. To test the system under varying temperature conditions that may occur in cheese manufacture.

4. To determine the effect of pH, rennet concentration, and calcium addition on poor coagulating late lactation milk.

5. To program a data acquisition system (equipped with hot wire, temperature and pH probes) to estimate the cut time of curd and to report deviations (pH levels, coagulation parameters, and temperature changes) from the expected norm during cheese manufacture.
PART I

CONFIGURATION OF A ZYMATE® ROBOT SYSTEM AND THE HOT WIRE SYSTEM FOR COAGULATION STUDIES
Abstract

The Zymate® II laboratory system (Zymark Co., Hopkinton, MA) was configured to prepare milk samples for coagulation tests with the hot wire system. The overall system, which included the laboratory robot, controller, and the power and event station, was configured specifically for coagulation studies. A hot wire probe, RTD (resistance temperature detector) probe, and a pH probe were used to monitor milk coagulation. The power and event station was used to turn the power supply of the hot wire probe on or off, which signaled the computer to commence or cease the collection of data.

Introduction

The Zymate® robotic system from Zymark consists of a robotic arm and multiple hand attachments that can be positioned and moved within 1mm in the three-dimensional reach of the arm. This robot was selected as a repeatable instrument for sample preparation and probe insertion for coagulation measurements. Since the robot also had the capabilities to turn power lines on and off, and to produce a varied voltage signal, the instrument could be used to signal a computer when data should be collected.

Objective

The objective was to design and build a laboratory data acquisition and control system using a Zymate® II robot to provide sample repeatability in the collection of milk pH, temperature, and coagulation curve parameters.

Materials And Methods

General operation. The Zymate® robot, Power and Event (P&E) station, printer, and other system resources were managed through the Zymate® Laboratory Controller (Figures 1 and 2). The robotic arm was the heart of the system (Figure 3) and was
configured similar to that of Yuan (1). It had a 60-cm reach, could move up and down, and rotate 370°. Detachable hands included with the system were used to grasp objects and dispense syringes. These hands could rotate 360° and were used for reagent addition and movement of samples from station to station. Movements of the robot and power and event controls were programmed with Easylab® software supplied by Zymark. The commands were sequential and sub-routines were permitted. Many of the commands involved moving to a specific location with three-dimensional coordinates. A training module was used to move the robotic arm to a specific location where coordinates were given a locational name used during programming. The P&E station permitted two alternating current (AC) lines to be turned on and off. A third AC line could be operated at variable voltage levels. Aside from the AC line control, the P&E controller had several analog-to-digital (AD) converters and switches for turning low current direct current (DC) lines on and off.

Coagulation tests. A modular station was assembled that would hold six 300-ml fleakers™ in an insulated water bath. Inlet and return spigots were built into the water bath so 2°C water could be continuously circulated through the bath. The station also contained culture and coagulant reservoirs. Easylab instructions (Appendix A) and data acquisition software (originally written in QuickBasic® 4.5) were written to prepare samples and collect the data.

At the beginning of each analysis, the robot attached the appropriate gripping hand (Figure 4) and removed the culture from the water bath (Figure 5). The culture moved to the stirring station where it was mixed to insure that all inoculations were uniform. The culture flask was returned and a milk sample was inoculated. The robot switched from the grip hand to the syringe hand (Figure 6) to complete the task. The inoculated milk sample moved to the stirring warming (Figure 7), station and the hot wire, pH, and temperature probes were automatically rinsed (Figure 8) and
Figure 1. Robotic system layout including robotic arm, controller, and personal computer.
Figure 2. Zymate® II laboratory controller.
Figure 3. Zymate® II robotic arm.
Figure 4. Attachment of grip hand to the robotic arm.
Figure 5. Culture is picked up from the ice bath and moved to the stir plate.
inserted into the sample (Figure 9). The robot turned on the power supply of the HWP, which signaled the PC to monitor the sample. The Zymate® robot was instructed to wait until it received a voltage increase from the DASCON-1 (Metrabite Corporation, Taunton, MA) board in the TeleVideo™ PC (TeleVideo™ Systems, Inc., Sunnyvale, CA). The TeleVideo™ was programmed to wait until the correct temperature was reached before signaling the robot to continue. Once the signal was given, the robot injected the coagulant into the milk sample. The P&E controller was used to stir the sample for 30 s and to wait for data collection. After data collection, the stirplate was turned on, and cutting and cooking of the curd were simulated. The probes were removed and rinsed, and the sample was returned to the ice bath. The process was repeated until all samples were automatically monitored.

**Data acquisition.** The TeleVideo™ PC was equipped with a DASCON-1 data acquisition board. The board has four analog-to-digital (AD) converters and two digital-to-analog converters (DAC). A current of 1 mA could also be supplied by the board for RTD excitation. The AD read voltages over a ±2.0475 V range with 12-bit plus sign precision (1 part in 4096). The AD converters were used to record voltages from the RTD temperature probe, the pH probe, and HWP. The DAC’s were used to signal the Zymate® P&E controller that the correct temperatures were obtained. A Hewlett-Packard 6216C (Hewlett-Packard Co., Salt Lake City, UT) power supply was used to supply 300 mA constant current required for the HWP.

**Conclusions**

The system worked well by providing consistent sampling and monitoring of milk coagulation. Communication between the Zymate® controller and the PC compatible computer was simple, but effective. By supplying power to the hot wire probe, the Zymate® controller could initiate or terminate data acquisition, and the PC...
Figure 6. Syringe hand.
Figure 7. Placement of a milk sample in the stirrer warmer.
Figure 8. Probe being rinsed before insertion into milk.
Figure 9. Probes in sample during coagulation measurement.
compatible computer could indicate the stage of data collection by sending varied voltage signals to the Zymate® controller.

Temperature, pH, and coagulation data obtained during each test were saved as a text data file on the hard drive of the PC compatible computer.

References

PART II
DETECTION OF COAGULATION AND CURD
FIRMING RATE BY THE HOT WIRE METHOD
Abstract

A vat monitoring system was configured with a hot wire coagulation probe, pH probe, and an RTD temperature probe. After addition of rennet (.015, .010, and .0075 rennet units per ml), milk substrate coagulation time was determined with this system and four other methods (Formagraph, Sommer and Matsen apparatus, Brookfield® LVT viscometer, and an Omnispec™ bioactivity monitor). The coagulation detection times measured by the hot wire system were measured at maxima of the first and second derivatives. Coagulation time was detected in the following ascending order: hot wire probe (maximum second derivative), Omnispec™, hot wire probe (maximum first derivative), Sommer and Matsen method, Formagraph, and Brookfield® LVT viscometer. The maximum rate of temperature change (~T' max) of the hot wire probe was measured during coagulation of milk substrate treated with decreasing levels (.030, .015, .0075, .0038, and .0019 rennet units/ml) of rennet. For each treatment, ~T' max was significantly different (P < .05), which indicated that rate of temperature change can be used to detect differences in curd firming rates.

Introduction

Effective monitoring and control of cheese manufacture assures consistent cheese quality and yield. Though temperature and sometimes pH are recorded, few automated systems are currently used to monitor curd formation during commercial manufacture of cheese. Information obtained by monitoring curd development could be used to compensate for differences in milk coagulability and culture performance. For example, if milk is heated, homogenized, or stored improperly, a weak curd could form after coagulation (9). Salt balance, pH, enzyme coagulant type, and season of the year can also affect milk clotting and curd firmness (3, 8, 12). Such variations in milk for cheese-making prevent manufacturers from cutting curd at a uniform firmness. If the coagulum
is cut too soon, fat and protein fines may be lost into the whey (2), which reduces profits. If it is cut too late, the curd could shatter or moisture removal could be impeded (19), resulting in high-moisture cheese and solids losses.

The need for an acceptable coagulation monitoring system exists, in order to monitor large enclosed vats for cheese manufacture that are tended by fewer workers where automated timing and control systems are in use. Much shorter heal times are also necessary in deeper vats because increased hydrostatic pressure promotes matting of the curd. For these reasons, measurement or assessment of the coagulum is necessary to maintain yields or prevent losses.

Many different instruments have been developed to monitor coagulation (4, 6, 7, 10, 11, 12, 13, 14, 15, 18, 19). Most monitor resistance to a shear force on the developing curd. Some follow a change in light absorbance, reflectance, or light scattering. However, each instrument has its own inherent problems and has not been completely applied in the cheese industry.

The hot wire method uses the principle of heat diffusion to determine viscometric properties of a fluid. The ambient fluid temperature (T_s) is measured by an RTD probe, and the estimated surface temperature of the hot wire probe (HWP) (Snow Brand Milk Products Co, Ltd., Saitama, Japan) is calculated (16). If the heat transfer characteristics of a fluid are known (or can be approximated), then kinematic viscosity can be calculated. Any difference between the ambient sample temperature and the surface of the HWP can be used as a relative indicator of the viscometric properties of the fluid. Fluids with higher viscosities develop slower convective currents around the hot wire, and consequently, the temperature difference (ΔT) between the HWP and T_s is greater. The purpose of this research was to determine the ability of the hot wire method to monitor coagulation and curd formation in experimental tests.
Objectives

1. To compare coagulation time determined by the hot wire instrument with other instruments commonly used to detect coagulation time.

2. To determine the ability of the hot wire system to detect differences in curd firmness.

Materials And Methods

Milk was prepared by reconstituting 12 g of low-heat nonfat dry milk (NDM) in 100 ml of .01M CaCl2 as described by Berridge (1). The milk was then stored for 18 h at 4°C to allow complete rehydration of NDM and equilibration of Ca++ salts. The milk substrate was warmed to 30°C and tempered for 30 min prior to tests to permit equilibration of milk salts at the test temperature. Double strength purified calf rennet [157 rennet units per milliliter (RU/ml)] was obtained from the New Zealand Cooperative Rennet Co., Ltd. (Eltham, New Zealand). Rennet dilutions were prepared with distilled, deionized water and kept on ice during experimentation.

Hot wire method. A HWP (6, 7, 16) and appropriate equations for determining the internal wire temperature and the estimated surface temperature of the probe were obtained from Snow Brand Milk Co. Ltd. The probe was heated by a Hewlett-Packard 6216C constant current power supply (Hewlett-Packard Co., Salt Lake City, UT), which maintained a 300-mA current. A DASCON-1 digital-to-analog computer interface board (Metrabite Corporation, Taunton, MA) with a resolution of 12 bits plus sign over a ±2.0475 volt range was used to measure the voltage across the probe during tests. The ambient sample temperature was measured with a 100-Ω RTD temperature probe. The RTD temperature probe, HWP, and pH probe were inserted vertically through a hard rubber stopper (Figure 10). Software was written in Visual Basic™ and Quick Basic™.
Figure 10. Bench-top hot wire data acquisition setup.
to record $\Delta T$ (temperature difference between ambient sample temperature and HWP temperature), $\Delta T'$ (rate of change of $\Delta T$, °C/s), $\Delta T''$ (rate of change of $\Delta T'$ °C/s²) and coagulation time (determined as the time when $\Delta T'$ is maximum ($CT'$), and the time when $\Delta T''$ is maximum ($CT''$)). The stopper containing the probes was placed vertically in the opening of a 300-ml fleaker™. During small-scale tests, the temperature was maintained by placing the fleaker™ in a constant temperature water bath. The temperature values of the HWP, $T_s$, $\Delta T$, and pH were collected at 5-s intervals. Typical $\Delta T$, $\Delta T'$, and $\Delta T''$ curves obtained during coagulation are shown in Figure 11.

Rolling bottle method. The apparatus described by Sommer and Matsen (20) was used for coagulation detection at 30°C. Approximately 50 ml of milk substrate (20), with added rennet, was added to a prewarmed (30°C) 125-ml wide mouth bottle, which was placed in the Sommer and Matsen apparatus within 1 min of rennet addition. Coagulation time was recorded as the time from rennet addition to the appearance of coagulum flecks in the milk film on the inner surface of the rolling bottle.

Viscosity measurement. Viscosity was measured by a procedure similar to that of Richardson et al. (17). Approximately 100 ml of milk substrate with added rennet was added to a pre-warmed (30°C) 25-mm x 150-mm test tube set in a 30°C water bath. A Brookfield® LVT (Brookfield Engineering Laboratories, Inc., Stoughton, MA) helipath viscometer was fitted with a #1 T-bar spindle. The spindle was submerged into the substrate; the helipath stand was turned on, and the viscometer speed was set at 60 rpm. Measurement commenced within 1 min of rennet addition. Coagulation time was determined by extrapolating from the increasing viscosity line to the time it crossed the baseline.

Formagraph method. Coagulation detection by the Formagraph (Type 11700, Foss Food Technology Corp., Eden Prairie, MN) followed the procedure of McMahon a
ΔT: Temperature difference between the hot wire probe surface and the sample.
ΔT': Rate of change of ΔT; °C/s
ΔT'': Rate of change of ΔT''; °C/s²

Figure 11. Typical hot wire and derivative curves.
nd Brown (14) with a few adaptations. For coagulation detection time comparisons, approximately 10 ml of milk substrate with added rennet was added to a pre-warmed (30°C). Formagraph sample well and sample recording began 1 min after rennet addition. Coagulation time was determined as the time from rennet addition to the point where the baseline began to increase (Figure 12). Curd firming rates ($k_{10}$ and $k_{20}$) were measured as the time from gelation to the point where widths of 10 and 20 mm were obtained on the Formagraph.

**Omnispec™ method.** Coagulation was measured by diffuse reflectance with a controlled-temperature Omnispec™ bioactivity monitor prototype (Wescor Inc., Logan, UT). Milk samples were placed in a 96-well polystyrene microwell plate that was inserted into the machine within 1 min after rennet addition. Temperature was maintained at 30°C and the $L^*$ value (Hunter measurement of reflected light intensity from dark to light, on a scale from 0 to 100) was measured at 15-s intervals as described by Yuan (21). Coagulation detection time was determined at the time of the first peak in the diffuse reflectance curve.

**Coagulation detection comparison.** A randomized block design experiment repeated three times was conducted to compare coagulation time detectability of five instruments (hot wire, Formagraph, Sommer and Matsen apparatus, Brookfield® LVT viscometer, and Omnispec™) at three rennet levels (.015, .010, and .0075 RU/ml of substrate). The milk substrate was warmed to 30°C and allowed to equilibrate for a minimum of 30 min prior to rennet addition. Rennet solution was prepared by diluting double strength (157 RU/ML) pure calf rennet to 1/200, 1/300 and 1/400 in distilled water. Rennet solution was added (2% wt/wt) to 700 ml of milk substrate, which was then mixed for 30 s. Appropriate volumes from the original 700 ml were placed in each instrument (within 1 min from coagulant addition) at 30.0 ± .5°C for coagulation time determination.
Figure 12. Diagram of coagulation and curd firmness as a function of time as recorded with the Formagraph.
Relative curd strength. Ability of the HWP to differentiate between substrate coagulated with various levels (.03, .015, .0075, .0038, and .0019 R.U./ml of substrate) of rennet was studied using a completely randomized statistical design. Rennet solution was prepared by diluting double strength (157 RU/ML) pure calf rennet to 1/100, 1/200, 1/400, 1/800, and 1/1600 in distilled water. Samples of Berridge substrate (300 ml) in fleakers™ were prepared in triplicate and warmed to 30°C and held for a minimum of 30 min before rennet addition (2% v/v). After rennet addition, samples were inverted gently at approximately 2-s intervals for 45-s and placed in a 30°C water bath, after which the HWP and temperature probe were placed vertically in the sample, and data were recorded at 5-s intervals for 25 min.

Results and Discussion

Coagulation detection. Coagulation time detection by the various instruments is illustrated in Figure 13. The relationship between coagulation time and 1/rennet concentration was linear ($R^2 = .99$) at the concentrations of rennet used. By dividing the coagulation times from the various dilutions by 2, 3, or 4, a composite measurement was obtained for statistical analysis. The analysis of variance was performed using the model $y \ (\text{coagulation time} / \text{rennet dilution}) = \text{block method}$. Tukey's Studentized Range Test was used to differentiate mean groupings. The time after enzyme addition required to reach the coagulation point measured by each method was $CT'' < CT'_o < CT' < CT_{sm} < CT_f < CT_v$, where $CT'_o = \text{Omnispec}^{TM} \ \text{coagulation time (diffuse light reflectance)}, \ \text{CT}_{sm}$ = coagulation time by the Sommer and Matsen method, $CT_f = \text{Formagraph coagulation time}, \ \text{and CT}_v = \text{Brookfield}^{®} \ \text{viscometer coagulation time}$. The hot wire detection time $CT'$ did not differ significantly from the Sommer and Matsen method (Table 1), which is still recognized as the standard method by the industry. Coagulation was detected
Figure 13. Measurement of coagulation time by selected methods. (Error bars represent standard error of the mean.)
TABLE 1. Estimated coagulation time comparison among methods (standardized to .03 RU/ml by dividing coagulation time by 2, 3, or 4).

<table>
<thead>
<tr>
<th>Method</th>
<th>min (coagulation time / rennet dilution)</th>
<th>Std dev¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot wire, 2nd derivative</td>
<td>4.04</td>
<td>.03ª</td>
</tr>
<tr>
<td>Omnispec™</td>
<td>4.10</td>
<td>.05ªªº</td>
</tr>
<tr>
<td>Hot wire, 1st derivative</td>
<td>4.20</td>
<td>.06ªª</td>
</tr>
<tr>
<td>Sommer and Matsen</td>
<td>4.26</td>
<td>.04ªªººº</td>
</tr>
<tr>
<td>Formagraph</td>
<td>4.36</td>
<td>.02ªºª</td>
</tr>
<tr>
<td>Viscometer</td>
<td>4.49</td>
<td>.08ª</td>
</tr>
</tbody>
</table>

¹ Means grouped with different letters (a, b, c, d, e, ) are significantly different at α = .05

significantly earlier by the hot wire (CT") and Omnispec™ method than by other methods that detect coagulation by resistance to a physical shear force.

Relative curd strength. Curd firming rate of milk substrate is decreased when the substrate is treated with decreasing levels of rennet (15), as in this experiment. The ΔT curves (Figure 14) during the coagulation of milk substrate (coagulated with different levels of rennet) are superimposed so that data are presented from 3 min before CT' through 4 min after CT'. Data were analyzed using the following completely random one-way ANOVA models:

\[
\Delta T \text{ (at } CT') = \text{ rennet level}
\]

\[
\Delta T \text{ (at } CT' + 4 \text{ min}) = \text{ rennet level}
\]

\[
\Delta T' \text{ max} = \text{ rennet level.}
\]

The ΔT values used in the analysis of variance could not be used to distinguish any of the applied treatments, but ΔT' max values (Figure 15) for each level of coagulant used were significantly different. Because the curd firming rate is slower for each sample treated with decreasing rennet levels, the hot wire ΔT' max value can be used as an
indicator of relative initial curd firming rates. Once a gel forms, and heat transfer from the HWP occurs by conduction only, curd firmness differences cannot be measured by the hot wire system. This conclusion is also supported by data observed by Hori (5). Hori measured the thermal conductivity of skim milk, with differing milk solids levels, before and after rennet treatment. In each treatment, the thermal conductivity of the curd differed only slightly from that of the skim milk. Increased curd firmness due to higher solids could not be supported by his data. This information can also be used to explain why $\Delta T$ values (of milk substrate with differing firming rates) were not significantly different 4 min after coagulation detection in this study. These results differ from later work by Hori (6), who graphically illustrated differences in relative kinematic viscosity (measured by a hot wire system) of rennet-treated skim milk. Even so, the hot wire data appear to be most useful for the determination of coagulation time and initial curd firming rate.

**Conclusions**

1. The hot wire instrument worked well as an objective method for determining coagulation time ($CT'$ and $CT''$) by enzyme coagulants. When compared with other instruments, the hot wire detected coagulation ($CT'$) after a diffuse reflectance method (Omnispec) and before methods that record resistance to physical shear. Coagulation determination between the hot wire ($CT'$) and the industry standard Sommer and Matsen rolling bottle method were not significantly different.

2. The hot wire method also provided a relative curd firming rate value ($\Delta T'$ max) that could be used on-line during cheese manufacture to detect differences during enzymic milk coagulation. These data could be used as part of an optimization scheme, or to signal gross errors (such as no rennet addition) in coagulation of milk for cheese
Figure 14. Mean hot wire values ($\Delta T$) during curd formation of milk substrate with varied rennet additions. (Error bars represent standard error of the mean.)
Figure 15. Relative curd firming rate indicated by differences in the maximum values (mean ± sd) of the first derivative curves (ΔT' max).
manufacture. This method would also be well suited for use in closed vats where curd inspection may be difficult.

References


PART III
CORRELATION OF HOT WIRE VALUES
WITH FORMAGRAPHS VALUES FOR
PREDICTION OF CURD CUT TIME
Abstract

Correlation of hot wire viscometric measurements with Formagraph firmness data (for renneted milk) was studied using a $2^5$ factorial design. Treatment levels included protein (2.75% and 3.25%), added calcium (0% and 0.01%), pH (6.54 or 6.28), fat (0% and 3.25%), and rennet (.03 or .015 RU/ml of milk). Rennet coagulation tests were run simultaneously using the hot wire instrument (with pH and temperature sensors) and a Formagraph. All five main factors significantly ($P < .05$) affected the time required to reach the cut time for rennet-treated milks determined on the Formagraph k20.

Significant interactions ($P < .05$) included rennet x pH, rennet x protein, and fat x protein. Coagulation time, maximum first derivative value, and selected area values of the first derivative curve of the hot wire data were used together with milk composition variables for correlation (step-wise regression) with Formagraph cut time firmness. Linear correlations ($R^2$ of .78 to .94) with coagulation and composition data were obtained in relating time from rennet addition to cutting.

Other tests were conducted to determine how hot wire values are affected by external heating and cooling of milk during enzymic coagulation. Although the baseline temperature difference between the hot wire probe temperature and the ambient sample temperature was affected by the treatments, the first derivative maximum value (indicative of curd firming rate) was not significantly affected by the temperature treatments.

Coagulation time was also detectable during acid coagulation, but the rate of pH drop during acid coagulation affected the curd firming rate measured by the hot wire system.

The system was also used to monitor simulated cheese manufacture in 300-ml batches and Cheddar cheese manufacture in 300-kg pilot-scale batches. Hot wire values could also be used to determine the cut time, healing period, and agitation of the curd as well as coagulation time and relative curd firming rate.
Introduction

Greater control of cheese manufacture could lead to increased yields and improved quality. As cheese making on an industrial level increases, individual cheese makers with small, well-tended vats are replaced by industrial systems that maximize facilities and materials. Infrared instruments are used in-line to measure and adjust fat-to-protein ratios in cheese milk (1). Similarly, producer incentive programs reward dairy farmers for reduced bacterial loads and low somatic cells in milk. Because of incentive programs, analysis instruments, and defined culture characteristics, a more uniform supply of milk to the vat generally allows cheese to be made on a fixed time schedule when time is a premium factor in processor costs.

Curd strength should be evaluated during cheese manufacture to accommodate differences in milk coagulability. Often, cheese makers examine curd by pressing the forming curd between their fingers. This method may be inconsistent in small vats and difficult, impractical, or impossible when large, closed vats are used. Therefore, the curd may be cut at a firmness far from optimal.

Many factors not related to microbial status of milk affect the quality of milk for cheese manufacture. Seasonal differences in diet of the cattle (28), stage of lactation (21, 6), milk pH (22, 26), casein micelle size (5), and protein profile of milk (2, 23) can all affect milk coagulation. Even milk from different quarters of the same cow can coagulate differently (24). For these reasons, coagulation properties and cheese yields have been investigated (20).

Changes in storage and handling of milk for cheese making are also important. These include heat treatment, homogenization, storage time, temperature (3, 7, 12), and coagulant type (14, 18). If no effort is made during cheese manufacture to compensate for milk of differing coagulability, then yields and quality of cheese made in an automated or tightly scheduled cheese plant may vary.
Previous research has shown that yields from cheese made from milk with intermediate and fast firming rates resulted in increased yields (2, 20). Riddle-Lawrence and Hicks (27) showed that when cheese was cut at relatively high curd strength, dry matter and cheese yield increased. But yield also increased when curd was cut at the lowest tension if the healing time were extended. Bynum and Olson (4) observed increased yields with no significant increase in moisture when curd was cut at higher tensions. These results were disputed by Mayes and Sutherland (15). Significant yield reductions did not occur even if curd was cut at 60% of the optimal cut time (16). When the cut time was extended from 116 to 200% of the optimal time, their estimations of economic increases due to increased yields were off-set by increased moisture and loss of quality (15).

One objective of this study was to compare measurements of the hot wire system (8, 9, 10) to those of the Formagraph, under conditions of varying milk composition (protein, fat), with calcium chloride additions, pH differences, and varied rennet additions to determine which measurements provide an optimal cut-time prediction. Other objectives included the observations of the selected hot wire measurements during coagulation with varying temperature treatments, during acid coagulation, and during Cheddar cheese manufacture. Determination of a cut window during cheese manufacture would aid cheese makers in optimizing scheduling, coagulant, and CaCl2 additions while also detecting poor coagulation performance of milk.

Objectives

1. To determine the portions of the hot wire curve that are most related to curd firmness and compare these data with Formagraph curd firmness values obtained during enzyme coagulation.
2. To test the ability of the hot wire to measure coagulation time and curd firming rate during acid coagulation.
3. To test the system under conditions that would simulate differences in temperature between the ambient milk temperature and the vat wall.
4. To measure coagulation, cutting, and healing of curd in bench-scale and pilot-scale cheese manufacture.

Materials and methods

Comparison of hot wire values with formagraph values. Milk was obtained from the Utah State University dairy herd and skimmed with a commercial separator. Its basic composition was determined by infrared analysis (Multispec™ IR, Foss Food Technology Corp., Eden Prairie, MN). The milk was batch pasteurized at 64°C for 30 min, cooled to 51°C, and ultrafiltered to 1.8x concentration (by volume reduction). Retentate and permeate were recombined to produce milk samples of the desired protein concentrations (2.75% or 3.25%). Infrared analysis was used again to verify protein concentration. Duplicate milk samples of differing protein, fat, calcium, and pH levels were prepared. Sodium azide (.02%, wt/wt) was added to each sample to retard spoilage, and samples were stored at 4°C. Calcium chloride was added to half of the samples at .02% (wt/wt). The pH of each sample was adjusted with dropwise addition of 2.1 N lactic acid or 1.0 N NaOH to either 6.95 or 6.58 (±.01) at 5°C. The pH was adjusted cold to prevent localized precipitation and protein denaturation. After warming to 30°C for 30 min or more the two pH levels were 6.54 (± .05) and 6.28 (± .06). Cream (35% butter fat) was added cold (no addition or 3.25%) and dispersed by inverting the sample at 1-s intervals for 1 min prior to warming and rennet addition. The added fluid milk in the cream was taken into account for Ca adjustment.
Samples were warmed to 30°C at least 30 min prior to rennet addition. Rennet solution was prepared by diluting double strength (157 RU/ML) pure calf rennet (New Zealand Cooperative Rennet Co., Ltd., Eltham, New Zealand) to 1/100 or 1/200 in distilled water. The rennet was then added to each sample individually at 2% v/v [.03% or .015% RU/ml substrate], based on the nonfat portion of the milk sample. Each sample was inverted at 1-s intervals for 30 s and measured simultaneously at 30°C on a Formagraph and by the hot wire method. Coagulation time (CT₁), as described by McMahon and Brown (19), and cut time (CT₂₀), which was the time from enzyme addition until a width of 20 mm was reached on the Formagraph curd firmness tracing, were determined. Data from the hot wire were collected at 5-s intervals. Software was written in Microsoft® Visual Basic™ and Quick Basic™ to determine temperature difference between the surface of the HWP and the ambient sample temperature (ΔT), first derivative (ΔT'), maximum ΔT' value (ΔT' max.), and areas under specific parts of the ΔT' curve (Figure 16). Coagulation time determined by the HWP was defined as the time from rennet addition to ΔT' max. The first derivative value (ΔT') was calculated by measuring the change in temperature of a moving average of three data points over a 30-s interval (°C/30s). The resulting value was divided by 30 (°C/s). By recording the rate of change over a larger time interval, and by using a moving average of three data points a smoother ΔT' curve was obtained.

Results were analyzed by GLM ANOVA procedures (SAS Institute, Inc., Cary, NC) in a randomized block factorial design repeated twice. Least squares linear regression was done using the REG or STEPWISE procedure of SAS.

Effects of sample temperature changes on hot wire values during coagulation. A completely randomized factorial experiment with three milk substrate temperatures (20, 30, or 40°C) and three water bath temperatures (20, 30, or 40°C) was designed to determine the effects of sample temperature changes during monitoring of enzymic
Figure 16. Typical hot wire and derivative curves.

\[ \Delta T: \text{Temperature difference between hot wire probe surface and the sample.} \]
\[ 1\text{st derivative value (}\Delta T'\text{): Rate of change of }\Delta T: {^\circ}\text{C/s} \]
\[ 2\text{nd derivative curve: Rate of change of }\Delta T': {^\circ}\text{C/s}^2 \]
\[ \text{Area 1: area under the 1st derivative 50 s prior to clot time.} \]
\[ \text{Area 2: area under the 1st derivative 25 s after to clot time.} \]
\[ \text{Area 3: area under the 1st derivative 25 s to 75 s after to clot time.} \]
coagulation of milk on hot wire values. These temperature changes simulated variations of temperature differences between milk for Cheddar cheese manufacture and the sides of a heated cheese vat. Rennet solution was prepared by diluting double strength (157 RU/ML) pure calf rennet to 1/100 in distilled water. Milk substrate (12g NFDM in 100 ml .01M CaCl2) was warmed to 20, 30, or 40°C and held at that temperature for a minimum of 30 min before 2% v/v rennet addition (.031 RU/ml of milk). After rennet addition and mixing for 45-s, the milk was placed in a 20°, 30°, or 40°C water bath and the temperature probe and HWP were inserted. Data were collected at 5-s intervals.

Acid coagulation. Milk substrate was prepared in triplicate by adding 12 or 8 g NDM per 100 ml of deionized water, or per 100 ml of .01M CaCl2 solution. The milk substrate was prepared 18 h prior to testing and was held at 30°C for 30 min before testing. Four percent (wt/wt) glucono-δ-lactone (GDL) was added to 300 ml of milk substrate, which was mixed by inverting at 5-s intervals for a period of 2 min. The HWP, temperature, and pH probes were inserted in the milk and data were collected at 5-s intervals. This procedure was repeated, and approximately 10 ml of the acidified substrate was added to a Formagraph for physical monitoring of curd formation.

Monitoring cheese manufacture with the hot wire. Cheddar cheese manufacture (four replications) was simulated in 300-ml fleakers™ using milk substrate coagulated with rennet (.03 RU/ml of milk substrate) at 30°C. The HWP, temperature, and pH probes were inserted vertically into the milk 1 min after enzyme addition. The curd was cut after 15 min and allowed to heal for 5 min and then stirred for 2 min. In a pilot-scale test, Cheddar cheese was made with four 300-kg batches of pasteurized whole milk from the USU dairy herd. The HWP, pH, and temperature probes were inserted vertically into a cheese vat, 2 in. from the vat wall, prior to coagulant addition, where they remained until after cooking. Data were collected at 5-s intervals.
Results and Discussion

Comparison of hot wire values with Formagraph values. When the Formagraph recording is 20mm wide it approximates the firmness at which cheese curd is cut. This time (CT₂₀) was selected as the physical firmness reference to which the hot wire values were compared. The analysis of variance for CT₂₀ is shown in Table 2. All five main treatments (protein, pH, calcium, fat, and rennet level) and three or their two-way interactions had a significant effect on the time required to reach cutting firmness. No three-way or four-way interactions were significant. Similar analyses of variance were conducted on the clot times recorded by both instruments and on ΔT’ max from the hot wire. The significant effects from these analyses of variance are shown in Tables 3, 4, and 5. Compared to the CT₂₀ analysis, fewer treatments significantly affected clot time or ΔT’ values. These results agree with those of Jen and Ashworth (11) that curd final strength is more sensitive to treatment differences than is curd clot time.

Limitations of the hot wire probe. As the viscosity of milk increases during coagulation there is a phase transformation from a liquid to a gel, and the convective flow around the HWP decreases. During this time, the rate of increase of ΔT is related to the rate of viscosity change. However, once the gel sets, the increase in curd firmness has little effect on the change in ΔT. The hot wire temperature should approach equilibrium at the same rate, regardless of curd firmness, once the milk ceases to flow past the HWP and heat is transferred by conduction only. Hori (8) has shown that thermal conductivity of skim milk curd is not greatly affected by curd firmness and differs only slightly from that of skim milk. Increasing solids content, therefore increasing curd firmness, does not cause increases in thermal conductivity of the skim milk curd relative to skim milk of the same solids level.

Our results confirmed this assumption. Larger ΔT’ max values did correlate with CT₂₀ (Figure 17). But, the value of ΔT at time equivalent to CT₂₀ was greater when
TABLE 2. Factorial analysis of variance of cut time (CT20) approximation.

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RP=rep, REN=Rennet, PH=pH, CA=calcium, PROT = protein
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RP=rep, REN=rennet, PH=pH, CA=calcium, PROT = protein
### TABLE 4. Factorial analysis of variance of Formagraph coagulation time (CT₁).

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Error 31

RP = rep, REN = rennet, PH = pH, CA = calcium, PROT = protein
TABLE 5. Factorial analysis of variance of hot wire coagulation time (CT).

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RP=rep, REN=rennet, PH=pH, CA=calcium, PROT = protein
CT_{20} was larger (Figure 18). This implies that the value of ΔT at cut time is more of a function of time rather than curd firmness.

Integration of areas under selected portions of the derivative curve also produced correlations with curd firming rate when analyzed by stepwise regression (Figures 17, 19, and 20). A correlation of $R^2 = .74$ was obtained between Area 2 (from 50 s before CT to CT) under the derivative curve and CT_{20} (Figure 19), and the correlation between the ΔT' max and CT_{20} was $R^2 = .75$ (Figure 17). However, the time to reach ΔT' max (CT) was more closely related ($R^2 = .89$) than any of the other values relating specifically to the curd firming rate (Figure 20). The clustering of CT vs. CT_{20} data are mostly due to the effects of rennet and pH. Clot time and curd firming rates obtained during the beginning of curd formation were not as sensitive to differences in milk characteristics as curd rigidity measurements from the Formagraph, but CT and ΔT' max can be used for cut time estimations. The correlation is good between clot time and clot-to-cut time (19) since the clot time is affected by many factors that alter milk coagulation and curd formation. These include pH, temperature, coagulant level, and calcium level (29).

Olson (25) indicated that the increase in gel rigidity followed first-order kinetics and that longer clot times were followed by a slower approach to maximum rigidity.

The average ratio of rennet-to-clot vs. clot-to-cut time in this study was 1:92, and is illustrated in Figure 20. Kowalchyk and Olson (13) showed an approximate 1:9 relationship between clot time and clot-to-cut time when rennet was used as the coagulant. Chymosin and pepsin mixtures caused greater variance between the rennet-to-clot and clot-to-cut time measurements. Ustunol et al. (30) recorded variations in clot-to-cut time with eight different commercial coagulants with equally standardized RU/ml. The ratio of the clot time to the clot-to-cut time was nearly 1:1 with all the coagulants tested except for bovine pepsin where twice as much time was required to obtain cutting firmness.
Figure 17. Predicted cut time based on the hot wire first derivative maximum value ($\Delta T^\prime$ max.) vs. Formagraph cut time ($CT_{20}$). $y = 3.042 + 0.78x$
Figure 18. Increase in ΔT (above baseline) at CT_{20} (Formagraph cut time) vs. CT_{20}.
Figure 19. Predicted cut time based on area 2 of the first derivative curve vs. the Formagraph cut time (CT$_{20}$). $y = 3.54 + .739x$
Figure 20. Predicted cut time based on the hot wire coagulation time (CT) vs. the Formagraph cut time (CT20). $y = .95 + 1.92x$
Other compositional and treatment factors were included in multiple stepwise regressions since they were found to have a significant effect on the CT\(_{20}\) value from the analysis of variance. The effects of the hot wire data combined with the compositional changes are illustrated in Figures 21 to 23. By including the protein level and pH of the milk, correlations above R\(^2\) = .90 were obtained. This indicates that information obtained from the hot wire can be used individually, or in conjunction with other information, such as milk composition, to correlate with a physical curd firmness value.

Fat and calcium changes are not included in the regressions since their effect was minimal and was not significant in the regression analysis. Added calcium is known to have an effect on clot time and curd firming rate (17, 19, 22, 29). The effect is partially due to lowered pH caused by displaced H\(^+\) ions. The pH effect of calcium was minimized in this study by adjusting the pH after calcium addition. Calcium additions followed by pH adjustment seem to have a greater firming effect on milk that does not coagulate as readily or at lower rennet levels (unpublished data).

**Temperature changes.** In general terms, the hot wire value (ΔT) is related to the ability of a fluid to convect or conduct heat away from the HWP. As viscosity of a fluid such as milk increases, there is less convection, and therefore, ΔT also increases. However, convection currents can also be generated by forces external to the HWP (e.g., stirring, heating, and cooling of vats). Such heat transfer forces are not related to the temperature difference between the HWP and the surrounding milk. Such convection currents would tend to increase or decrease ΔT depending on whether the milk was cooled or heated.

At lower ambient temperatures (e.g., 20° C), as shown by ΔT' max, the coagulation rate is slower than at 40° C (Figure 24). This is expected because the enzymic phase of milk coagulation has a Q\(_{10}\) = 2, while the agglagation phase has a Q\(_{10}\)
Figure 21. Prediction of cut time based on curve area (sum of areas 1, 2, and 3), protein concentration, milk pH, and rennet concentration vs. the Formagraph cut time (CT_{20}). $Y = (-35.9 \times \text{sum area}_1 + 2 + 3) + (27.15 \times \text{pH}) - (5.76 \times \text{prot}) - (12.02 \times \text{ren}) - 115$. 

$R^2 = 0.92$
Figure 22. Prediction of cut time based on $\Delta T'$ max., protein concentration, milk pH, and rennet concentration vs. the Formagraph cut time ($CT_{20}$). $Y = ((-2.8 \times 10^4) \times \Delta T'_{\text{max}}) - (9.7 \times 10^4 \times \Delta T'_{\text{max}}^2) + (27.9 \times \text{pH}) - (4.88 \times \text{prot}) - (12.1 \times \text{ren}) - 62.6$

$R^2 = 0.94$
Figure 23. Prediction of cut time based on clot time, milk pH, and protein concentration vs. the Formagraph cut time (CT20). \( Y = (1.71 \times CT) + (6.52 \times pH) - (4.96 \times prot) - 24.62 \)

\( R^2 = 0.94 \)
The baseline \( \Delta T \) values differed by as much as 0.4 °C when external heat transfer was induced (i.e., bath water temperatures different than sample temperatures). However, unless this difference was very large (e.g., 20 °C), it had a minimal effect on \( \Delta T' \max \) readings. If milk at 40 °C were placed in a 20 °C water bath, \( \Delta T' \max \) decreased from 0.0058 °C/s to 0.0051 °C/s. Putting the same milk in a 30 °C water bath did not, however, cause any change in \( \Delta T' \max \). Conversely, putting 20 °C milk in a higher temperature water bath (30 °C or 40 °C) increased \( \Delta T' \max \) (Figure 24). Thus, while induced convection currents alter baseline \( \Delta T \), the rate of change of \( \Delta T \) is more dependent on transition of milk from a liquid to a gel. Even so, such large temperature differentials as used in this experiment are unlikely to be encountered in industrial-scale cheese making. For this reason, it was concluded that coagulation time and curd formation rate measured by a HWP would not be altered significantly by relatively minor differentials between jacket and milk temperatures during cheese manufacture.

Acid coagulation. Making the milk with only 8g NDM/100 ml produced a weak gel when it was acidified (Table 6). The Formagraph readings did not even reach 20 mm. It coagulated much faster than the 12g NDM/100 ml milk because, with less milk solids, it had less buffering capacity and acidification was faster (e.g., 0.045 pH units/min compared to 0.029 pH units/min). Consequently its \( \Delta T' \max \) values were higher. Adding calcium had an interesting effect. The milk was required to drop to a lower pH before it coagulated. The clot times in the milk prepared in 0.01 \( M \) CaCl\(_2\) were shorter because such milk substrates had an initial pH of 6.3. The milk substrates prepared with water started at about pH 6.7. In rennet coagulation, there is a progressive destabilization of casein micelles. The aggregation process begins as soon as enough \( \kappa \)-casein is hydrolyzed to destabilize the casein micelles. Then as more micelles are destabilized, there are eventually enough clusters of micelles formed to create a gel network. In
Figure 24. The effect of external heat transfer on hot wire $\Delta T'$ max values of rennet coagulated milk substrate at different temperatures (mean $\Delta T'$ ± sd).
TABLE 6. Acid coagulation of milk substrate.

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<td>WATER</td>
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¹ averaged over 3 min prior to coagulation
* A weak coagulum was formed and k20 and k30 were never achieved
Samples with different superscripts (within rows) are significantly different (P < .001)
contrast, when milk is acidified, all of the micelles undergo a change at the same time. Thus, when the pH has been sufficiently reduced, all of the micelles will become destabilized, and a gel is formed. Thus, in rennet coagulation $\Delta T^*$ max is a function of how quickly the micelles are being destabilized and being incorporated into the gel network. In acid coagulation, the interpretation of $\Delta T^*$ max is more complex. Increasing the acidification rate shortens the time before coagulation, but does not affect the pH at which coagulation occurs. Nor does it affect the curd firmness. With faster acidification, $\Delta T^*$ is increased.

Within each solids level, the rate of pH drop was similar and $\Delta T^*$ max was inversely related to Formagraph k20 as expected. Maximum curd firming rates ($\Delta T^*$ max) from the four treatments were significantly different ($P < .001$).

Therefore, information on the rate of pH change is necessary to determine whether or not the rate of curd formation is faster because of quicker acidification or because of differences in milk composition. Nevertheless, both coagulation time and curd firming rate can be monitored using the HWP during milk acidification such as in the manufacture of cottage cheese. These data suggest that coagulation time and curd formation rate can be collected by the hot wire during acid coagulation such as during cottage cheese manufacture. Other tests would need to be conducted to determine how to best integrate these types of data into a quality control and coagulation measurement system for acid coagulation of milk.

**Cheese manufacture.** Changes in $\Delta T$ during laboratory scale cheese manufacture (in 350-ml fleakers™) are shown in Figure 25. Coagulation time, cutting, healing, stirring, and rates of temperature change were all detectable by the hot wire system and could be included in a report generated by computer software. Similar results were seen in larger vat (300 kg) Cheddar cheese manufacture. These results indicate that the hot wire instrument is not only suitable for bench-top applications' usage, but also may be
Figure 25. Hot wire measurements ($\Delta T$) recorded during bench-top-scale cheese manufacture.
used in manufacturing-scale equipment to detect stirring, coagulation, and healing of cheese curd.

**Conclusions**

1. The hot wire instrument detected clot time at the time of the maximum rate of change of the hot wire curve ($\Delta T'_{\text{max}}$). Also, the average rise in temperature of the HWP 50 s prior to coagulation (Area 1) and the average rise in temperature 25 s after coagulation (Area 2) correlated with the curd firming rate data measured by the Formagraph. Final curd firmness could not be measured with the system, but, in this study, correlations of $R^2 = .71$ to $R^2 = .94$ were obtained when either the clot time or curd firming data from the hot wire were combined with pH and compositional data.

2. Acid coagulation can also be monitored, provided that the rate of pH drop is considered in firming estimations.

3. Vat conditions that generate forced convection on the hot wire probe minimally affected hot wire curd firming rate data ($\Delta T'_{\text{max}}$).

4. The hot wire data could be used to detect stirring (forced convection), coagulation, cutting of curd, and curd healing in bench-top and pilot-scale cheese manufacture. On-line monitoring of milk coagulation, along with pH and temperature data collection, could help cheese manufacturers control make procedures within their own specifications. These data show that an appropriate cut window can be established, thus offering a cheese maker the opportunity to vary the process within that window.

**References**


Hori, T. 1985, Objective measurements of the process of curd formation during rennet treatment of milks by the hot wire method. J. Food Sci. 50:911.


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PART IV
THE EFFECTS OF CALCIUM, pH, AND RENNET CHANGES
ON THE COAGULATION PROPERTIES
OF LATE LACTATION MILK
Abstract

Ten late lactation milk samples were selected based on their coagulation properties. Upon the addition of rennet, four samples coagulated well, and six samples coagulated poorly. Fat, protein, lactose, solids-not-fat, pH, and SDS-PAGE data were collected on skimmed samples. Calcium, pH, and coagulant levels were adjusted using a split-split plot factorial design. The pH of each sample was adjusted to 6.6, 6.4, or 6.2, and CaCl2 was added at .02, .01, or 0%. Rennet was added to samples at .031 and .062 rennet units/ml, and a Formagraph was used to record coagulation time, k10, k20, and relative curd firmness 30 min after adding rennet (a30). Each main treatment had a significant effect (P < .05) on each of the four coagulation parameters tested. Many of the interactions were also significant, including rennet x pH and calcium x pH. However, protein and lactose contents as well as protein bands and density differences shown by SDS-PAGE electrophoresis could not be correlated with milk coagulability.

Introduction

Coagulation properties of milk for cheese manufacture do not remain constant during the course of lactation (1, 2, 8, 12, 15). Seasonal changes (4, 13, 16) and dietary factors (14) can affect composition and enzymic coagulation of milk. Milk obtained during late lactation may produce a weak coagulum (3) not suitable for cheese manufacture. Okigbo et al. (9) showed that the stage of lactation had a significant effect on the rennet-coagulation time (CT), curd firmness, and estimations of cheese yield.

There are some indications that casein degradation is partially responsible for the poor coagulation performance of late lactation milk. In general, CT decreases with increased levels of β-casein and total casein (16). Poor coagulating milk was shown to have a higher content of γ-caseins and lower levels of κ-casein and β-casein than good-
Coagulating milk. Plasmin, a natural milk protease, increases during late lactation and has been suggested as a possible contributor to casein degradation (8).

Because of poor coagulation performance, late lactation milk is often combined with bulk milk representing all stages of lactation. Even so, blending of good and poor coagulating milk does not always insure milk suitable for cheese manufacture (8). In countries such as Australia and New Zealand, where cattle commence lactation according to seasons, mid-lactation milk may not be available for commingling with late lactation milk. Regester and Smithers (14) found a significant increase in the amount of glycomacropeptide in whey protein concentrate from the milk collected late in the Australian milking season. Although season and lactation stage are confounded in Regester and Smithers's study, increases in glycomacropeptide may indicate compositional differences of late lactation milk. The increase could also be related to a more complete hydrolysis of κ-casein before adequate curd formation.

Aside from blending milk samples, chemical adjustments can improve milk coagulability (8, 10). The purpose of this study was to elucidate the effects of calcium, pH, and rennet on individual good and poor coagulating milk samples from individual cows in late lactation.

**Objective**

The objective of these experiments was to determine the effects of pH, rennet concentration, and calcium addition, on poor coagulating late lactation milk.

**Materials and methods**

Selection of milk samples. Evening milk samples from 21 late lactation Holstein cows from the Utah State University dairy herd were tested for good and poor rennet coagulability according to the method of Okigbo et al. (11). Five samples considered to have poor rennet coagulability and five samples considered to have good rennet
coagulability were selected for further analysis. Approximately 1 wk after the initial test, 1500 to 2000 ml of milk were collected from a complete evening milking of each of the selected cows. Approximately 25 ml from each sample were taken for somatic cell count determination. The milk samples were then stored at 3°C for 24 h and the cream was siphoned from the top of each sample. After being mixed for 1 min, 20 ml from each of the skimmed samples were examined for good or poor rennet coagulability (11). Six samples coagulated poorly and four samples coagulated well. Approximately 100 ml of milk from each sample were separated for chemical analysis (protein, lactose, fat, and solids-not-fat), protein fractionation (SDS-PAGE), and pH.

**Chemical tests.** Milk samples were warmed to 30 ± 1°C and were held at that temperature for 30 min before the "initial" pH of each milk sample was measured with a pH meter (Orion Research Inc., Cambridge, MA). SDS-PAGE fractionation was performed in duplicate using a Phast™ System with PhastGel™ homogeneous 20 gels (Pharmacia, Uppsala, Sweden). Each sample (100 µl) was diluted to 1 ml with Tris (10 mM)-EDTA buffer (pH 8.0). The samples were further diluted by adding 300 µl of 10% SDS and 50 µl of β-mercaptoethanol. The samples were heated in a boiling water bath for 5 min and were then refrigerated. A tracking dye (3.0 µl of 4.5%, wt/v, bromophenol blue in distilled water) was mixed with the samples and .5 µl of the sample mixture was loaded on the gel. The samples were loaded on the anodic end of the gel at 250 V, 1.0 mA, 3.0 W, and 15°C at 0 Vh.

A 1% Coomassie Blue in 10% acetic acid solution was used for gel staining. The gels were destained with 9% methanol and 1% acetic acid solution in the development chamber of the Phast™ System. After further destaining (14 h) in fresh destaining solution, the gels were fixed in a solution of 10% glycerol and 10% acetic acid.
Basic composition (protein, lactose, fat, and solids-not-fat) was performed in duplicate using a Multispec™ infrared instrument (Foss Food Technology Corp., Eden Prairie, MN).

**Experimental design.** A split-split plot randomized block design (replicated twice) was used with varied pH levels, added calcium, and rennet additions to each of the milk samples. Milk from each sample was divided into nine partitions. CaCl₂ was added at .02, .01, or 0% followed by pH adjustment to 6.6, 6.4, or 6.2 at 3°C with 2.1 N lactic acid or 1.0 N NaOH while stirring. Sodium azide (.01%) and penicillin (10 IU/ml) were added as preservatives, and the samples were stored at 4 ± 1°C prior to testing. All samples were warmed to 30°C and held at that temperature for 30 min prior to coagulation tests. The pH was readjusted as previously to eliminate minor pH differences that occur during storage. Rennet solution was prepared by diluting double strength (157 RU/ML) pure calf rennet to 1/100, or 1/50 in distilled water. Coagulation time, k₂₀, and a₃₀ (6) were obtained after the addition of 2% v/v rennet solution (.03 RU/ml or .06 RU/ml) of the rennet solution from a Formagraph (Type 11700, Foss Food Technology Corp., Eden Prairie, MN) as described by McMahon and Brown (6).

**Statistical analysis.** Results from the Formagraph analysis were analyzed by PROC GLM procedures in a split-split-plot model randomized factorial design with two replications (SAS Institute, Inc., Cary, NC). Individual milk samples were used as the main plots, with calcium and pH adjustments as the first subplot and rennet additions as the second subplot.

**Results and Discussion**

**General composition.** The general characterization of the milk samples is shown in Table 7. The pH was generally higher in the poor coagulating samples as reported by Okigbo et al. (8), but samples 7 and 9 did not reflect those differences. Protein and total
solids percentage from the good and poor coagulating milk samples were similar and could not be used as an indicator of coagulability.

As expected, the days-in-milk production (DIM) had no general effect on the determination of initial coagulability since all cows were in milk production for over 220 d. Cow 4 was in milk production for a longer period than any of the other cows in this study, and her milk failed to coagulate in the initial tests.

Protein fractionation. Okigbo et al. (11) reported unidentified protein fractions and γ-casein peaks, which are generally derived from the breakdown of β-casein in poor coagulating milk samples. The SDS-PAGE gel containing each of the 10 samples is shown in Figure 26. Samples with higher somatic cell counts have γ-casein bands indicative of protein breakdown. No bands were observed that would be indicative of poor or good coagulating milk. Other studies (3), with milk samples that coagulated poorly, have shown that removal of smaller protein fragments increases milk coagulability. Other methods of protein characterization, such as hydroxyapatite chromatography (11) or FPLC (fast protein liquid chromatography), could yield differences that were not detected by SDS-PAGE fractionation.

Treatment effects on late lactation milk. All main treatments (pH, calcium, coagulant, and sample) had a significant effect on rennet coagulation time, $k_{20}$, and $a_{30}$ measurements of milk from individual cows used in this study (Tables 8, 9, and 10). Normally, the addition of calcium to milk is accompanied by a small drop in pH, which reduces electrostatic repulsions between micelles and increases $[Ca^{2+}]$ in solution (7). This effect was minimized since pH adjustments were performed after calcium addition. Divalent calcium also acts as a bridge between negative phosphate groups and negative protein side chains on the individual micelles, and aggregation is facilitated (7). A reduction in pH of normal milk also increases the activity of most coagulants and increases the calcium ion concentration in the milk (5). Therefore, it was not surprising
that many of the interactions were also significant. Significant interactions between the treatments and the individual milk samples are shown in Table 11.

When individual samples were compared, calcium addition did not significantly affect \( \text{CT}_f \) of the good coagulating milk. In contrast, \( \text{CT}_f \) was shortened for all poor coagulating samples with calcium addition. Chymosin and pH interactions appeared to have more effect on good coagulating samples than on the poor coagulating samples.

These results are similar to previous studies that included calcium chloride effects on bulk milk with good coagulation properties. Graphs containing data from each of the individual samples (coagulated with .03 RU/ml rennet) are shown in Figures 27 to 32. Coagulation times for initially good and poor coagulating samples are shown in Figures 27 and 28. Samples that initially coagulated poorly show a greater response to pH and calcium treatments than samples that initially coagulated well. The milk sample from cow 4 coagulated only after treatments with .02% calcium chloride and after lowering the pH to 6.2. Initially poor coagulating milk samples were more sensitive to calcium and pH treatments as examined by Formagraph \( k_{20} \) values (Figures 29 and 30), and samples 2, 5, and 6 were particularly sensitive to calcium x pH interactions. Both calcium additions and pH treatments were required to shorten the \( k_{20} \) time to similar levels to those of good coagulating samples. Milk sample 4 did not respond well enough to the applied treatments to produce sufficient curd strength for cheese manufacture.

In actual cheese manufacture, pH adjustment in poor coagulating samples can be accomplished by allowing culture growth for a period of time prior to coagulant addition, but this procedure also increases the chance of phage attack. Calcium additions and use of increased coagulant levels can be used to improve coagulation. But, excessive coagulant may lead to bitterness or off-flavors in cheese. Some coagulants and cultures may also interact to produce bitter peptides. The use of excessive calcium chloride has
TABLE 7. Milk characterization.

<table>
<thead>
<tr>
<th>Milk Sample</th>
<th>% BF</th>
<th>Protein</th>
<th>Lactose</th>
<th>SNF²</th>
<th>SCC³</th>
<th>pH@30°C</th>
<th>DIM⁴</th>
<th>Initial Coagulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.59</td>
<td>3.30</td>
<td>4.99</td>
<td>9.26</td>
<td>85</td>
<td>6.48</td>
<td>247</td>
<td>GOOD</td>
</tr>
<tr>
<td>2</td>
<td>.66</td>
<td>2.78</td>
<td>4.09</td>
<td>7.68</td>
<td>340</td>
<td>6.73</td>
<td>277</td>
<td>POOR</td>
</tr>
<tr>
<td>3</td>
<td>.55</td>
<td>3.10</td>
<td>4.42</td>
<td>8.44</td>
<td>374</td>
<td>6.76</td>
<td>222</td>
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</tr>
<tr>
<td>4</td>
<td>.18</td>
<td>3.30</td>
<td>4.80</td>
<td>9.74</td>
<td>21</td>
<td>6.70</td>
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<tr>
<td>5</td>
<td>.66</td>
<td>3.81</td>
<td>4.97</td>
<td>9.24</td>
<td>36</td>
<td>6.67</td>
<td>231</td>
<td>POOR</td>
</tr>
<tr>
<td>6</td>
<td>.36</td>
<td>3.30</td>
<td>4.47</td>
<td>8.57</td>
<td>265</td>
<td>6.77</td>
<td>293</td>
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</tr>
<tr>
<td>7</td>
<td>1.22</td>
<td>3.15</td>
<td>4.89</td>
<td>8.95</td>
<td>41</td>
<td>6.59</td>
<td>275</td>
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</tr>
<tr>
<td>8</td>
<td>.92</td>
<td>3.45</td>
<td>4.97</td>
<td>9.43</td>
<td>78</td>
<td>6.60</td>
<td>255</td>
<td>GOOD</td>
</tr>
<tr>
<td>9</td>
<td>.49</td>
<td>3.30</td>
<td>4.84</td>
<td>9.11</td>
<td>224</td>
<td>6.69</td>
<td>266</td>
<td>GOOD</td>
</tr>
<tr>
<td>10</td>
<td>3.35</td>
<td>3.19</td>
<td>4.82</td>
<td>8.85</td>
<td>502</td>
<td>6.62</td>
<td>281</td>
<td>GOOD</td>
</tr>
</tbody>
</table>

¹% BF = % butterfat of skimmed samples, SNF² = solids not fat, SCC³ = somatic cell count of full fat sample, DIM⁴ = days in milk production
<table>
<thead>
<tr>
<th>Initial Coagulability</th>
<th>Poor</th>
<th>Good</th>
<th>Poor</th>
<th>Poor</th>
<th>Good</th>
<th>Good</th>
<th>Poor</th>
<th>Poor</th>
<th>Poor</th>
<th>Good</th>
<th>Milk control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Milk control</td>
<td>3</td>
<td>1</td>
<td>7</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

Figure 26. PAGE Gel of good and poor coagulating late-lactation milk samples.
TABLE 8. Split-split plot analysis of variance. Significant effects on the Formagraph clot time of individual cow milk with good and poor coagulating properties.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>MS</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
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</tr>
<tr>
<td>Sample (S)</td>
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<td>7765.3</td>
<td>153.56</td>
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</tr>
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<td>Error a [Rep*S]</td>
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<td>50.53</td>
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<td></td>
</tr>
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<td>2145.16</td>
<td>61.03</td>
<td>.0001</td>
</tr>
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<td>12946.0</td>
<td>368.33</td>
<td>.0001</td>
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<td>2.66</td>
<td>.0389</td>
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<td>232.64</td>
<td>6.62</td>
<td>.0001</td>
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<tr>
<td>S*pH</td>
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<td>550.80</td>
<td>15.67</td>
<td>.0001</td>
</tr>
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<td>S<em>Ca</em>pH</td>
<td>36</td>
<td>151.81</td>
<td>4.32</td>
<td>.0001</td>
</tr>
<tr>
<td>Error b [Rep<em>S(Ca</em>pH)]</td>
<td>80</td>
<td>35.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rennet (Ren)</td>
<td>1</td>
<td>3027.6</td>
<td>140.07</td>
<td>.0001</td>
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<td>68.44</td>
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<td>.32</td>
<td>.7296</td>
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<td>Ren*pH</td>
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<td>317.63</td>
<td>14.69</td>
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<td>Ren<em>Ca</em>pH</td>
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<td>28.69</td>
<td>1.33</td>
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<td>.84</td>
<td>.6445</td>
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<tr>
<td>S<em>Ren</em>Ca*pH</td>
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<td>32.41</td>
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<td>.0635</td>
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<td>Error c</td>
<td>90</td>
<td>21.61</td>
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<td></td>
</tr>
</tbody>
</table>

Ren=Rennet, Ca=calcium, S=sample
TABLE 9. Split-split plot analysis of variance. Significant effects on the a30 of individual cow milk with good and poor coagulating properties.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>MS</th>
<th>F Value</th>
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<td>Rep</td>
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<td>Sample (S)</td>
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<td>6352.18</td>
<td>75.3</td>
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<td></td>
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<td>Calcium (Ca)</td>
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<td>1460.28</td>
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<td>5985.5</td>
<td>204.82</td>
<td>.0001</td>
</tr>
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<td>Ca*pH</td>
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<td>137.33</td>
<td>4.70</td>
<td>.0019</td>
</tr>
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<td>S*Ca</td>
<td>18</td>
<td>182.07</td>
<td>6.23</td>
<td>.0001</td>
</tr>
<tr>
<td>S*pH</td>
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<td>380.38</td>
<td>13.02</td>
<td>.0001</td>
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<td>117.02</td>
<td>4.00</td>
<td>.0001</td>
</tr>
<tr>
<td>Error b [Rep<em>S(Ca</em>pH)]</td>
<td>80</td>
<td>29.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rennet (Ren)</td>
<td>1</td>
<td>219.34</td>
<td>13.46</td>
<td>.0004</td>
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<td>S*Ren</td>
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<td>113.42</td>
<td>6.96</td>
<td>.0001</td>
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<td>Ren*Ca</td>
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<td>76.92</td>
<td>4.72</td>
<td>.0112</td>
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<td>Ren*pH</td>
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<tr>
<td>S<em>Ren</em>pH</td>
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<td>23.05</td>
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<tr>
<td>S<em>Ren</em>Ca*pH</td>
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<td>23.19</td>
<td>1.41</td>
<td>.0965</td>
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<tr>
<td>Error c</td>
<td>90</td>
<td>16.30</td>
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<td></td>
</tr>
</tbody>
</table>

\[\text{Rep=S*Ca*pH(\text{Ren})}\]

Ren=Rennet, Ca=calcium, S=sample
TABLE 10. Split-split plot analysis of variance. Significant effects on the curd firming rate ($k_{20}$) of individual cow milk with good and poor coagulating properties.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>MS</th>
<th>F Value</th>
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<td></td>
</tr>
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<td>Calcium (Ca)</td>
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<td>4455.39</td>
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<tr>
<td>pH</td>
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<tr>
<td>Ca*pH</td>
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<td>.0001</td>
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<td>S*pH</td>
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<td>S<em>Ca</em>pH</td>
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<td>342.71</td>
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</tr>
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<td>Error b [Rep<em>S(Ca</em>pH)]</td>
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<td>Rennet (Ren)</td>
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<td>2.39</td>
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Ren=Rennet, Ca=calcium, S=sample
TABLE 11. Significant ($P < .05$) treatment and interaction effects on the clot time, $a_{30}$, and $k_{20}$ values of individual good and poor coagulating late lactation milk samples.

<table>
<thead>
<tr>
<th>Milk</th>
<th>Ca</th>
<th>pH</th>
<th>Chymosin</th>
<th>Ca*P</th>
<th>Ca*Chy</th>
<th>pH*Chy</th>
<th>Ca<em>P</em>Chy</th>
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<td>b</td>
<td>c</td>
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<td>c</td>
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<td>Good initial coagulation</td>
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<td>a</td>
<td>c</td>
<td>c</td>
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<td>c</td>
</tr>
</tbody>
</table>

$\text{a} =$ coagulation time significant ($P < .05$)  
$\text{b} =$ $k_{20}$ significant ($P < .05$)  
$\text{c} =$ $a_{30}$ significant ($P < .05$)
Figure 27. Formagraph cut time (CT<sub>20</sub>) of initially poor coagulating late lactation milk after CaCl<sub>2</sub> additions and pH adjustment.
Figure 28. Formagraph cut time (CT20) of initially good coagulating late lactation milk after CaCl2 additions and pH adjustment.
Figure 29. Coagulation time (CTf) of initially poor coagulating late lactation milk after CaCl2 additions and pH adjustment.
Figure 30. Coagulation time (CTr) of initially good coagulating late lactation milk after CaCl₂ additions and pH adjustment.
Figure 31. Curd firmness ($a_{30}$) of initially poor coagulating late lactation milk after CaCl$_2$ additions and pH adjustment.
Figure 32. Curd firmness ($a_{30}$) of initially good coagulating late lactation milk after CaCl$_2$ additions and pH adjustment.
Conclusion

Adjusting pH, adding calcium, and increased rennet usage can improve the coagulation of some poor coagulating late lactation milk, but not all. Since late lactation milk may have a higher pH than normal milk, pH adjustment may bring the coagulation properties within the normal range. Some samples may require added CaCl_2 or increased rennet addition, while other samples will still remain unsuitable for cheese manufacture. However, the root cause of poor coagulation in late lactation milk samples remains unresolved.

References


PART V

PROTOTYPE SOFTWARE DEVELOPMENT AND
APPLICATION IN CHEESE MANUFACTURE
Abstract

Software was written to record hot wire, pH, and temperature data during cheese manufacture. Specifications were included to recognize vat agitation and several phases of cheese manufacture, including coagulation, cutting of the curd, and curd healing. Data collected during cheese manufacture could also be compared to data collected earlier during previous cheese manufacture.

Introduction

Visual Basic™ is a newer version of Basic that contains programming tools and forms that access the features of Microsoft® Windows™. Most code written in Visual Basic™ is event oriented and is accessed only when specific events occur. These events include such actions as mouse movements, key clicks, pressed button icons, etc. Events that need to be repeated at specific time intervals are accessed through a timing event. The code in the timing event is accessed only when a selected timing interval elapses.

To simplify data acquisition, hardware companies provide dynamic link libraries (DLL) that are accessible through Windows™. The DLL's allow data acquisition requests to be obtained with simple one or two lines of code. By combining Visual Basic™ features and DLL's, software could be written to access and calculate data in real time.

Objective

The objective was to program a data acquisition system (equipped with hot wire, temperature, and pH probes) to estimate the cut time of curd and to report deviations (pH levels, coagulation parameters, and temperature changes) from the expected norm during cheese manufacture.

Materials and methods

Data acquisition software were written in Microsoft® Visual Basic™ 1.0 for the
Windows™ environment. The vat monitoring software begins by showing a title page and software name (Figure 33). The title page is closed and an option window (Figure 34) is opened when the mouse is clicked on the screen. Normal conditions can be monitored or data from previous runs can be accessed and graphed. If normal conditions are selected, then the main screen is displayed (Figure 35). File names and descriptions are keyed in, and a new run is started by clicking with the mouse on the "start run" button. Current data are displayed in four graphic picture windows (Figure 36), and real-time graphs (Figures 37 and 38) can be displayed on the screen. Double clicking on the graphs or picture windows brings up a screen that is used to change the axis scale (Figure 39). Running statistics, which include time, temperature, pH, and coagulation values, can be displayed while data are being collected (Figure 40). The time of events such as rennet addition, coagulation, and cutting is printed to the screen as the events occur (Figures 41 and 42). The pH, temperature, and coagulation values are also included. These data can be compared on-line with values from normal cheese manufacture. A similar screen (Figure 43) can also be displayed when data are read from a file. The software measures the height of the hot wire curves and first derivative curves to determine when events such as coagulation or cutting can occur. Cut time estimations are currently based on the curd firming rate, or on the coagulation time. The equation used for cut time estimation based on curd firming rate is: \[-(7.98 \times 10^3) \times (\Delta T'_{\text{max}}) + 57.62.\]

A 1:1 ratio from rennet-to-clot to clot-to-cut is used when coagulation time is used for estimations. The height of the first derivative curve also is used to provide a relative indicator of the curd firming rate. A subroutine was also written to calculate the first derivative maximum value area under the hot wire derivative curve at the end of each run. These data are saved to a file and can be used for user analysis.
Figure 33. Opening screen and credits of vat monitoring system software.
Figure 34. Vat monitoring start-up option window.
Figure 35. Main operating screen.
Figure 36. Main operating screen with graphical indicators of current monitoring.
Figure 37. Real-time graph of milk coagulation.
Figure 38. Real-time graph of milk coagulation, cutting, and healing of cheese curd.
Figure 39. Option window for changing axis scale.
Figure 40. Display of data values and running conditions during real-time monitoring.
Figure 41. Display of data values updated after coagulation.
Figure 42. Display of data values updated after cutting.
Figure 43. Display of data values after data are retrieved from a file.

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>TEMP.</th>
<th>MAX</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLOT TIME</td>
<td>6.58</td>
<td>6.32</td>
<td>30.73</td>
</tr>
<tr>
<td>CUT TIME</td>
<td>11.67</td>
<td>6.32</td>
<td>30.85</td>
</tr>
<tr>
<td>HEAL END</td>
<td>21.58</td>
<td>6.32</td>
<td>30.43</td>
</tr>
</tbody>
</table>
Conclusions

1. The software recorded temperature, pH, and hot wire values.

2. The system was programmed to estimate the curd cut time based on maximum first derivative and clot time values.

3. The system was programmed to report differences from normal values in temperature, pH, and hot wire values (including first derivative maximum value, clot-time) during cheese manufacture.
GENERAL SUMMARY AND CONCLUSIONS

Summary

The Zymate® II robot system was configured for milk sample preparation to be used with a compatible data collection system. A TeleVideo™ PC was configured with a DASCON-1 data acquisition board to obtain pH, coagulation, and temperature data. The machines communicated to allow sample preparation and data collection during coagulating of milk. Although the hot wire probe (HWP) coagulation system was successful in recording coagulation and initial curd firming rates, the system did not adequately record curd firmness data after a curd gel formed and prior to curd cutting. For this reason the robot system was not used for subsequent data collection.

The data acquisition system was configured for use in small and large vats. Not only was the system compatible with agitation, but the HWP could detect stirring, coagulation, and healing of the curd. Therefore, the software was programmed to save critical time, pH, and temperature data (rate of change and values) during important periods during manufacture. These data could be compared to normal or average data from previous manufacture of cheese.

Coagulation time detection and curd firming rate data were obtainable by the system. Coagulation time determination was similar in value and precision to laboratory test methods, including the rolling bottle method, viscometry, and Formagraph methods. Temperature differentials between milk and surrounding environment that would generate convection currents did not significantly affect firming rate ($\Delta T'$ max) data.

The HWP curd firming rate data were then used for correlation with data from an instrument that could detect firming rates and firmness values (Formagraph). Correlations of $R^2 = .78$ to .94 were obtained when HWP data and compositional data were used for correlations with the Formagraph data. This showed that the HWP system
can be used to aid cut time predictions that can allow a manufacturer to more fully optimize ingredient and timing steps during manufacture of cheese. The HWP would also detect major problems in a vat such as failure to add a critical ingredient such as coagulant or starter culture.

Further tests were completed on good and poor coagulating late lactation milk to determine if additional ingredients or treatments could improve coagulation. Increased rennet levels, lower pH, and CaCl₂ additions all improved coagulation. Some samples responded to minimal treatment while other sample did not respond to combinations of all treatments.

Prototype software was written to collect pH, temperature, and coagulation data during manufacture of cheese. The software was programmed to illustrate graphically what was happening in the cheese vat and to record curd firming data and rates of change of pH and temperature during phases of cheese making. The software was also programmed to estimate a cut time based on HWP coagulation time and curd firming rate data.

**Conclusions**

The hot wire probe fills a valuable need for cheese manufactures. Hot wire data have been shown to correlate with curd firmness measurements, and can be used as a tool to determine a cut "window" during cheese manufacture. Increased yields may or may not be realized, depending on how well the manufacturer currently guesses the cut window without the aid of an objective measurement. Coagulation measurement in closed vats would also help the manufacturer because access to the vats is limited, and subjective curd firmness measurement is also difficult to ascertain. Certainly, defective or slow coagulating batches could be identified before subsequent vats are prepared with the same coagulant or milk. This would also hold true for acid production when pH is
continuously monitored. A manufacturer could also use the hot wire as a tool to permit reduced coagulant and rennet additions, or to cut curd at an earlier time, and still be assured that a sufficiently firm gel has formed. Premature cutting of curd could also be avoided if coagulant is omitted. On a more sophisticated approach, hot wire data could be combined with other data such as somatic cell counts, bacterial counts, pH (at various stages during processing), temperature profiles, culture changes, and subjective evaluation with multiple regression and correlation techniques to further optimize cheese manufacture. Data regarding pH, calcium, rennet, fat, and protein obtained in this research can also be used to help cheese manufacturers adjust for differences in milk.
Appendix A

Vat Monitoring Application Software Code
' ********** GLOBAL CONSTANTS ARE SET

Global Const TRUE = -1
Global FILE$(6)
Global countdat(4)
   Global DEGC(10), SLOPETESTT(720), DIO%(4)
   Global DTS(720), RCOEF(10), TH(720), TIMES(720)
   Global RTD(720), filename$(2), PH(720)

Global ftimes(720), frtd(720), fdts(720), fph(720), fxmax, interval
Global timer1count, timer2count
Global Const FALSE = 0
Global B, HPRUN' COUNTER AND RUN INDICATOR

Global Const MODAL = 1
Global Const WILDCARD$ = "*.csv"

Global FullFilePath As String
Global FileSelected As Integer

Global AboutStr As String
Global Const xmax = 5000
Global Const ymax = 5000

Global UNIT, GRFSUM' (0 To 4) As Integer
Global DEFDER$, VGRAF(20)'GRAPHS TO VIEW
Global FERM1$(4) 'filenames for current saved run types
Global MAXRUNOFF(4), NOCHGOFF(4)
Global TIMERUN(8), der1(720)

Global SLOPEMAXB, TIMECUTB, TIMEHEALB, RENTIMEB,
CUTCHECKS, HEALCHECKS$
Global MAXCHECK, TIMEPLUS, COUNTA, COUNTB, countc, TIMEMAX
Global TIMEMAXTEMP, TIMEMAXPH, TIMECUT, ESTCUT, SLOPETEST
Global TIMECUTTEMP, TIMECUTPH, TIMEHEAL, TIMEHEALTEMP,
SLOPEMAX
Global TIMEHEALPH, HEATRATE, N, U, DTSMAX,
MAXSTUFFSTARTED
Global MT, POSRTA, POSDTA, PCHK4, PCHK3, PCHK2, PCHK1
Global COUNTB3, COUNTB2, COUNTB1, POSDT, POSRT
Global HR0, HR1, HR2, C0, C1
Global tics(4), tempyb(4), xb(4), phyb(4), dtsYB(4), der1YB(4), rtdYB(4), ENZ3YB(4)
Global GRAF$(4)
Declare Function GETFREESPACE Lib "Kernel" (ByVal wFlags As Integer) As Long
Global value1(4, 10), value2(4, 10), value3(4, 10)
Global Y1RANGE(IO), Y2RANGE(4)
Global Y1MAX(4), Y2MAX(4), Y1MIN(4), Y2MIN(4), TIMESQZED(4)
Global hours(4), GRF, HCOUNT(4), amp
Global FORM5STATS, INC 'USED IN TESTING WITHOUT ACTUAL DAC

Global Const BLACK = &H0&
Global Const RED = &HFF&
Global Const GREEN = &HFF00&
Global Const YELLOW = &HFFFF&
Global Const BLUE = &HFF0000
Global Const MAGENTA = &HFF00FF
Global Const CYAN = &HFFFF00
Global Const WHITE = &HFFFFFF
Global Const GRAY = &HCOCOCO

** THIS IS THE START FORM WITH NAME AND
*** BOXED PICTURE FORM1

Sub Form_Load ()
FORM1.Scale (0, 0)-(5000, 5000)
FORM1.Show
DBBOXFORM FORM1, 10, 10, 4980, 4980, GRAY
BOXFORM FORM1, 1000, 1200, 3000, 1800, GRAY
BOXFORM FORM1, 3000, 4400, 1000, 375, GRAY

CURRENTX = 1300: CURRENTY = 1900
FONTBOLD = TRUE
FONTSIZE = 18
FORECOLOR = black
Print "VAT MONITOR SYSTEM"
CURRENTX = 1315: CURRENTY = 1915
FONTBOLD = TRUE
FONTSIZE = 18
FORECOLOR = yellow
Print "VAT MONITOR SYSTEM"

CURRENTX = 2305: CURRENTY = 2605
FONTBOLD = TRUE
FONTSIZE = 9
FORECOLOR = white
Print "version 1.0 by Michael LeFevre"
CURRENTX = 2300: CURRENTY = 2600
FONTBOLD = TRUE
FONTSIZE = 9
FORECOLOR = BLUE
Print "version 1.0 by Michael LeFevre"

CURRENTX = 3050: CURRENTY = 4500
FONTBOLD = FALSE
FONTSIZE = 9
FORECOLOR = BLUE
Print "CLICK TO CONTINUE"

End Sub

Sub Form_Click ()
Load FORM2
FORM2.Show
End Sub

'***** FORM TO SELECT RUN TYPE AT 2ND FORM TO SHOW
'****FORM2
Dim slct 'FOR SELECTION OF OPTION

Sub Option1_Click (INDEX As Integer)
slct = INDEX '*** PICKS ONE OF 4 START OPTIONS
End Sub
Sub Command1_Click ()
Select Case slct
Case 0
    form3.Show
    form2.Hide
Case 1

Case 2
GETFILE.Show
form2.Hide 'SELECT AN OPTION FORM
Case 3
End Select
End Sub

Sub Form_Load ()
y1max(1) = 7: y1min(1) = 3
y2max(1) = 40: y2min(1) = 15
y1max(2) = 4: y1min(2) = 2
y2max(2) = 40: y2min(2) = 15
y1max(3) = 7: y1min(3) = 3
y2max(3) = 40: y2min(3) = 15
y1max(4) = 4: y1min(4) = 0
y2max(4) = 1: y2min(4) = -1

Load ut1grf
Load ut2grf
Load ut3grf
Load ut4grf
FORM1.Hide
form2.Show
BOXFORM form2, 540, 1230, 3600, 2000, gray

End Sub

Sub Command2_Click()

    TITLE$ = "TERMINATE PROGRAM?"
    NONAM$ = "Are you sure you want to quit the program?"

    TYP% = 3 + 16 + 256: MSG$ = NONAM$
    tst = MsgBox(MSG$, TYP%, TITLE$)

    If tst = 6 Then
        Unload form2
        Unload FORM1
    End If
End Sub

'*** THIS IS THE MAIN FORM YOU SEE DURING
'*** DATA COLLECTION AND MONITORING +FORM3

Sub Timer1_Timer()
    'If countdat(1) = 0 Then U1CLICK
    countdat(1) = countdat(1) + 1
    Call getdat 'has averages max min etc.

    '** THIS IS THE HEART OF DATA COLLECTION
    '** AND ANALYSIS

    'GET DATA
    'CALC
    'LINIERIZE
    'OUT OF BOUNDS MESSAGES
End Sub

Sub Timer2_Timer()
Call U1CLICK
Call U2CLICK
'TUPDATE GRAPHS

End Sub

Sub Command1_Click()

    c = 1
    FILENAMES$(c) = OUTPUTFILE.TEXT
    DESCRIP$ = TEXT1.TEXT
    FILENAMRL$ = FILENAME$(c) + WRITECOUNT1$ + ".dat"
    outintfile$ = INTFILE.TEXT
    Open FILENAMRL$ For Append As #2
    'form3.Hide
    timer1.ENABLED = TRUE
    timer2.ENABLED = TRUE
    OUTPUTFILE.ENABLED = FALSE
    INTFILE.ENABLED = FALSE
    TEXT1.ENABLED = FALSE
    COMMAND1.ENABLED = FALSE
    COMMAND2.ENABLED = FALSE
    COMMAND5.ENABLED = TRUE
    HPRUN = TRUE
    LABEL4(0).VISIBLE = TRUE
    LABEL4(1).VISIBLE = TRUE
    picture1.VISIBLE = TRUE
    picture2.VISIBLE = TRUE
    picture3.VISIBLE = TRUE
    picture4.VISIBLE = TRUE
    For N = 0 To 3
        LABEL5(N).VISIBLE = TRUE
        LABEL6(N).VISIBLE = TRUE
        LABEL7(N).VISIBLE = TRUE
        LABEL8(N).VISIBLE = TRUE
    Next N

End Sub

Sub Command2_Click()

Unload FORM3
form2.Show

End Sub

Sub Form_Load()
    FORM3.WINDOWSTATE = 2 'MAXIMIZED
For N = 0 To 3
  LABEL5(N).VISIBLE = FALSE
  LABEL6(N).VISIBLE = FALSE
  LABEL7(N).VISIBLE = FALSE
  LABEL8(N).VISIBLE = FALSE
Next N
SLOPMAXB = 0: TIMECUTB = 0: TIMEHEALB = 0: rentimeb = 0
MAXCHECK = 2.22
TIMEPLUS = 0: COUNTA = 0: COUNTB = 0: TIMEMAX = 0
TIMEMAXTEMP = 0: TIMEMAXPH = 0: TIMECUT = 0
TIMECUTTEMP = 0: TIMECUTPH = 0: TIMEHEAL = 0: TIMEHEALTEMP = 0
TIMEHEALPH = 0: HEATRATE = 0: DTSMAX = 0:
MAXSTUFFSTARTED = 0

MT = MTM * 60: POSRTA = 0: POSDTA = 0:
AMP = .3
GRAPHCHECK = 0: YAXISS = "LOW": YXIS = 3.2
PCHK4 = 0: PCHK3 = 0: PCHK2 = 0: PCHK1 = 0
COUNTB3 = 0
COUNTB2 = 0
COUNTB1 = 0

STOPFLAG$ = "N"

b = 2:
POSRT = 1: POSDT = -1

proprietary values hidden!!!
timer1.ENABLED = FALSE
timer2.ENABLED = FALSE
COMMAND5.ENABLED = FALSE
COMMAND1.ENABLED = TRUE

End Sub

Sub Command3_Click ()
UT1GRF.Show
UT2GRF.Show
End Sub

Sub Command5_Click()
    c = 1
    OUTPUTFILE.TEXT = ""
    TEXT1.TEXT = ""
    INTFILE.TEXT = ""
    Close #2
    'form3.Hide
    timer1.ENABLED = FALSE
    timer2.ENABLED = FALSE
    OUTPUTFILE.ENABLED = TRUE
    INTFILE.ENABLED = TRUE
    TEXT1.ENABLED = TRUE
    COMMAND1.ENABLED = TRUE
    COMMAND2.ENABLED = TRUE
    COMMAND5.ENABLED = FALSE
    HPRUN = FALSE
    LABEL4(0).VISIBLE = FALSE
    LABEL4(1).VISIBLE = FALSE
    picture1.VISIBLE = FALSE
    picture2.VISIBLE = FALSE
    picture3.VISIBLE = FALSE
    picture4.VISIBLE = FALSE
    For N = 0 To 3
        LABEL5(N).VISIBLE = FALSE
        LABEL6(N).VISIBLE = FALSE
        LABEL7(N).VISIBLE = FALSE
        LABEL8(N).VISIBLE = FALSE
    Next N
End Sub

Sub Command4_Click()
    GETFILE.Show
    form2.Hide 'SELECT AN OPTION FORM
End Sub

Sub Command7_Click()
    UT3GRF.Show
    UT4GRF.Show
End Sub

Sub STRTRUN_Click()
c = 1
FILENAME$(c) = OUTPUTFILE.TEXT
DESCR1P$ = TEXT1.TEXT
FILENAMRL$ = FILENAME$(c) + WRITECOUNT1$ + ".dat"
outintfile$ = INTFILE.TEXT
Open FILENAMRL$ For Append As #2
'form3.Hide
timer1.ENABLED = TRUE
timer2.ENABLED = TRUE
OUTPUTFILE.ENABLED = FALSE
INTFILE.ENABLED = FALSE
TEXT1.ENABLED = FALSE
COMMAND1.ENABLED = FALSE
COMMAND2.ENABLED = FALSE
COMMAND5.ENABLED = TRUE
HPRUN = TRUE
LABEL4(0).VISIBLE = TRUE "** IF ACTIVE MONITORING IS
SELECTED
LABEL4(1).VISIBLE = TRUE "** THEN THESE SHOW CURRENT
STATS
picture1.VISIBLE = TRUE
picture2.VISIBLE = TRUE
picture3.VISIBLE = TRUE
picture4.VISIBLE = TRUE
For N = 0 To 3
LABEL5(N).VISIBLE = TRUE
LABEL6(N).VISIBLE = TRUE
LABEL7(N).VISIBLE = TRUE
LABEL8(N).VISIBLE = TRUE
Next N
End Sub

Sub STPRUN_Click()
c = 1
OUTPUTFILE.TEXT = ""
TEXT1.TEXT = ""
INTFILE.TEXT = ""
Close #2
'form3.Hide
timer1.ENABLED = FALSE
timer2.ENABLED = FALSE
OUTPUTFILE.ENABLED = TRUE
INTFILE.ENABLED = TRUE
TEXT1.ENABLED = TRUE
COMMAND1.ENABLED = TRUE
COMMAND2.ENABLED = TRUE
COMMAND5.ENABLED = FALSE
HPRUN = FALSE
LABEL4(0).VISIBLE = FALSE
LABEL4(1).VISIBLE = FALSE
picture1.VISIBLE = FALSE
picture2.VISIBLE = FALSE
picture3.VISIBLE = FALSE
picture4.VISIBLE = FALSE

For N = 0 To 3
LABEL5(N).VISIBLE = FALSE
LABEL6(N).VISIBLE = FALSE
LABEL7(N).VISIBLE = FALSE
LABEL8(N).VISIBLE = FALSE
Next N

End Sub

Sub VIEWACT_Click()
UT1GRF.Show
UT2GRF.Show
End Sub

Sub LOADRUN_Click()
GETFILE.Show
form2.Hide 'SELECT AN OPTION FORM
End Sub

Sub VIEWGRF_Click()
UT3GRF.Show
UT4GRF.Show
End Sub

Sub SHOWALL_Click()
UT1GRF.Show
UT2GRF.Show
UT3GRF.Show
UT4GRF.Show
End Sub

Sub Picture1_DblClick()
GRF = 1
FORM9.LABEL2.CAPTION = "PH"
FORM9.LABEL5.CAPTION = "TEMPERATURE"
FORM9.TEXT1.TEXT = Str$(Y1MAX(1))
FORM9.TEXT2.TEXT = Str$(Y1MIN(1))
FORM9.TEXT3.TEXT = Str$(Y2MAX(1))
FORM9.TEXT4.TEXT = Str$(Y2MIN(1))
FORM9.Show 1
U1CLICK

End Sub

Sub Picture2_Click()
    GRF = 1
    FORM9.LABEL2.CAPTION = "PH"
    FORM9.LABEL5.CAPTION = "TEMPERATURE"
    FORM9.TEXT1.TEXT = Str$(Y1MAX(1))
    FORM9.TEXT2.TEXT = Str$(Y1MIN(1))
    FORM9.TEXT3.TEXT = Str$(Y2MAX(1))
    FORM9.TEXT4.TEXT = Str$(Y2MIN(1))
    FORM9.Show 1
    U1CLICK
End Sub

Sub Picture3_Click()
    GRF = 2
    FORM9.LABEL2.CAPTION = "hot wire"
    FORM9.LABEL5.CAPTION = "1ST DER"
    FORM9.TEXT1.TEXT = Str$(Y1MAX(2))
    FORM9.TEXT2.TEXT = Str$(Y1MIN(2))
    FORM9.TEXT3.TEXT = Str$(Y2MAX(2))
    FORM9.TEXT4.TEXT = Str$(Y2MIN(2))
    FORM9.Show 1
    U2CLICK
End Sub

Sub Picture4_Click()
    GRF = 2
    FORM9.LABEL2.CAPTION = "hot wire"
    FORM9.LABEL5.CAPTION = "1ST DER"
    FORM9.TEXT1.TEXT = Str$(Y1MAX(2))
    FORM9.TEXT2.TEXT = Str$(Y1MIN(2))
    FORM9.TEXT3.TEXT = Str$(Y2MAX(2))
    FORM9.TEXT4.TEXT = Str$(Y2MIN(2))
    FORM9.Show 1
    U2CLICK
End Sub

Sub EXPROG_Click()
    'CHECK TO SEE IF ANY PROGRAMS ARE ACTIVE
    End
End Sub

Sub Timer3_Timer()
    PICTURE6.Cls
PICTURE6.Print Date$, Time$
End Sub

Sub Command6_Click()
    TITLES$ = "TERMINATE PROGRAM?"
    NONAM$ = "Are you sure you want to quit the program?"
    TYP% = 3 + 16 + 256: MSG$ = NONAM$
    tst = MsgBox(MSG$, TYP%, TITLES)
    If tst = 6 Then
        Unload form2
        Unload FORM1
    End If
End Sub

Sub Command8_Click()
    FORM5STAT$ = "ON"
    FORM5.Show
End Sub

'** UNLOADS STATS FORM WHEN BUTTON IS CLICKED
'** ON STATS FORM5

Sub Command1_Click()
    FORM5STAT$ = "OFF"
    Unload FORM5
End Sub

'** SUBROUTINES FOR CALLING GRAPHS
'*********** graph 1 instructions

Sub Form_Resize()
    Call Form_Click
End Sub

Sub Form_Load()
    UT1GRF.HEIGHT = 2520 'SCREEN.HEIGHT * (1 / 4)
    UT1GRF.LEFT = 0
    UT1GRF.TOP = 1470
    UT1GRF.WIDTH = 6075'SCREEN.WIDTH * 1 / 4
    Call Form_Click
End Sub
Sub Form_Click ()
U1CLICK
End Sub

Sub Form_GotFocus ()
Call Form_Click
End Sub

Sub PGRF1_Click ()
PrintForm
End Sub

Sub Form_DblClick ()
GRF = 1
FORM9.LABEL2.CAPTION = "PH"
FORM9.LABEL5.CAPTION = "TEMPERATURE"
FORM9.TEXT1.TEXT = Str$(Y1MAX(1))
FORM9.TEXT2.TEXT = Str$(Y1MIN(1))
FORM9.TEXT3.TEXT = Str$(Y2MAX(1))
FORM9.TEXT4.TEXT = Str$(Y2MIN(1))
FORM9.Show I
U1CLICK
End Sub

Sub Check1_Click ()
U1CLICK
End Sub

/**************graph 2 instructions
Sub Form_Resize ()
    Call Form_Click
End Sub

Sub Form_Load ()
UT2GRF.HEIGHT = 2520'SCREEN.HEIGHT * (1/4)
UT2GRF.LEFT = 0'SCREEN.HEIGHT * (1/3)
UT2GRF.TOP = 4005'SCREEN.HEIGHT * (1/4)
UT2GRF.WIDTH = 6075'SCREEN.WIDTH * 1/4
    Call Form_Click
End Sub

Sub Form_Click ()
U2CLICK
End Sub

Sub Form_GotFocus ()
Call Form_Click
End Sub

Sub PGRF1_Click ()
PrintForm
End Sub

Sub Form_DblClick ()
GRF = 2
FORM9.LABEL2.CAPTION = "hot wire"
FORM9.LABEL5.CAPTION = "1ST DER"
FORM9.TEXT1.TEXT = Str$(Y1MAX(2))
FORM9.TEXT2.TEXT = Str$(Y1MIN(2))
FORM9.TEXT3.TEXT = Str$(Y2MAX(2))
FORM9.TEXT4.TEXT = Str$(Y2MIN(2))
FORM9.Show 1
U2CLICK
End Sub

Sub Check1_Click ()
U2CLICK
End Sub

'**********************graph 3 instructions

Sub Form_Resize ()
    Call Form_Click
End Sub

Sub Form_Load ()
UT3GRF.HEIGHT = SCREEN.HEIGHT * (1 / 4)
UT3GRF.LEFT = 0
UT3GRF.TOP = 2 * SCREEN.HEIGHT * (1 / 4)
UT3GRF.WIDTH = SCREEN.WIDTH * 1 / 4
    Call Form_Click
End Sub

Sub Form_Click ()
U3CLICK
End Sub

Sub Form_GotFocus ()
Call Form_Click
End Sub

Sub PGRF1_Click ()
PrintForm
Sub Form_DblClick()
    GRF = 3
    FORM9.LABEL2.CAPTION = "pH"
    FORM9.LABEL5.CAPTION = "TEMPERATURE"
    FORM9.TEXT1.TEXT = Str$(Y1MAX(3))
    FORM9.TEXT2.TEXT = Str$(Y1MIN(3))
    FORM9.TEXT3.TEXT = Str$(Y2MAX(3))
    FORM9.TEXT4.TEXT = Str$(Y2MIN(3))
    FORM9.Show 1
    U3CLICK
End Sub

Sub Check1_Click()
    U3CLICK
End Sub

'************ graph 4 instructions

Sub Form_Resize()
    Call Form_Click
End Sub

Sub Form_Load()
    UT4GRF.HEIGHT = SCREEN.HEIGHT * (1 / 4)
    UT4GRF.LEFT = 0
    UT4GRF.TOP = 3 * SCREEN.HEIGHT * (1 / 4)
    UT4GRF.WIDTH = SCREEN.WIDTH * 1 / 4
    Call Form_Click
End Sub

Sub Form_Click()
    u4click
End Sub

Sub Form_GotFocus()
    Call Form_Click
End Sub

Sub PGRF1_Click()
    PrintForm
End Sub

Sub Form_DblClick()
    GRF = 4
FORM9.LABEL2.CAPTION = "pH"
FORM9.LABEL5.CAPTION = "Temperature"
FORM9.TEXT1.TEXT = Str$(Y1MAX(4))
FORM9.TEXT2.TEXT = Str$(Y1MIN(4))
FORM9.TEXT3.TEXT = Str$(Y2MAX(4))
FORM9.TEXT4.TEXT = Str$(Y2MIN(4))
FORM9.Show 1
u4click
End Sub

Sub Check1_Click ()
u4click
End Sub

Sub Form_Resize ()
    Call Form_Click
End Sub

'** LOADS GRAPH 1 FORM UT1GRF

Sub Form_Resize ()
    Call Form_Click
End Sub

Sub Form_Load ()
UT1GRF.HEIGHT = 2520 'SCREEN.HEIGHT * (1 / 4)
UT1GRF.LEFT = 0
UT1GRF.TOP = 1470
UT1GRF.WIDTH = 6075 'SCREEN.WIDTH * 1 / 4
Call Form_Click
End Sub

Sub Form_Click ()
U1CLICK
End Sub

Sub Form_GotFocus ()
Call Form_Click
End Sub

Sub PGRF1_Click ()
PrintForm
End Sub
Sub Form_DblClick()
    GRF = 1
    FORM9.LABEL2.CAPTION = "PH"
    FORM9.LABEL5.CAPTION = "TEMPERATURE"
    FORM9.TEXT1.TEXT = Str$(Y1MAX(1))
    FORM9.TEXT2.TEXT = Str$(Y1MIN(1))
    FORM9.TEXT3.TEXT = Str$(Y2MAX(1))
    FORM9.TEXT4.TEXT = Str$(Y2MIN(1))
    FORM9.Show 1
    U1CLICK
End Sub

Sub Check1_Click()
    U1CLICK
End Sub

'** LOADS GRAPH 2 FORM UT2GRF

Sub Form_Resize()

    Call Form_Click
End Sub

Sub Form_Load()
    UT2GRF.HEIGHT = 2520' SCREEN.HEIGHT * (1/4)
    UT2GRF.LEFT = 0'SCREEN.HEIGHT * (1/3)
    UT2GRF.TOP = 4005' SCREEN.HEIGHT * (1/4)
    UT2GRF.WIDTH = 6075'SCREEN.WIDTH * 1/4

    Call Form_Click
End Sub

Sub Form_Click()
    U2CLICK
End Sub

Sub Form_GotFocus()
    Call Form_Click
End Sub

Sub PGRFl_Click()
    PrintForm
End Sub

Sub Form_DblClick()
    GRF = 2
    FORM9.LABEL2.CAPTION = "hot wire"
    FORM9.LABEL5.CAPTION = "IST DER"
    FORM9.TEXT1.TEXT = Str$(Y1MAX(2))
    FORM9.TEXT2.TEXT = Str$(Y1MIN(2))
End Sub
FORM9.TEXT3.TEXT = Str$(Y2MAX(2))
FORM9.TEXT4.TEXT = Str$(Y2MIN(2))
FORM9.Show 1
U2CLICK
End Sub

Sub Check1_Click ()
U2CLICK
End Sub

*** LOADS GRAPH 3 FORM UT3GRF

Sub Form_Resize ()

    Call Form_Click
End Sub

Sub Form_Load ()
UT3GRF.HEIGHT = SCREEN.HEIGHT * (1 / 4)
UT3GRF.LEFT = 0
UT3GRF.TOP = 2 * SCREEN.HEIGHT * (1 / 4)
UT3GRF.WIDTH = SCREEN.WIDTH * 1 / 4

    Call Form_Click
End Sub

Sub Form_Click ()
U3CLICK
End Sub

Sub Form_GotFocus ()
    Call Form_Click
End Sub

Sub PGRF1_Click ()
PrintForm
End Sub

Sub Form_DblClick ()
GRF = 3
FORM9.LABEL2.CAPTION = "pH"
FORM9.LABEL5.CAPTION = "TEMPERATURE"
FORM9.TEXT1.TEXT = Str$(Y1MAX(3))
FORM9.TEXT2.TEXT = Str$(Y1MIN(3))
FORM9.TEXT3.TEXT = Str$(Y2MAX(3))
FORM9.TEXT4.TEXT = Str$(Y2MIN(3))
FORM9.Show 1
U3CLICK
End Sub

Sub Check1_Click ()
U3CLICK
End Sub

'** LOADS GRAPH 4 FORM UT4GRF

Sub Form_Click ()
    Call Form_Click
End Sub

Sub Form_Load ()
    UT4GRF.HEIGHT = SCREEN.HEIGHT * (1 / 4)
    UT4GRF.LEFT = 0
    UT4GRF.TOP = 3 * SCREEN.HEIGHT * (1 / 4)
    UT4GRF.WIDTH = SCREEN.WIDTH * 1 / 4
    Call Form_Click
End Sub

Sub Form_Click ()
    u4click
End Sub

Sub Form_GotFocus ()
    Call Form_Click
End Sub

Sub PGRF1_Click ()
    PrintForm
End Sub

Sub Form_DblClick ()
    GRF = 4
    FORM9.LABEL2.CAPTION = "Hot wire value"
    FORM9.LABEL5.CAPTION = "1st derivative"
    FORM9.TEXT1.TEXT = Str$(Y1MAX(4))
    FORM9.TEXT2.TEXT = Str$(Y1MIN(4))
    FORM9.TEXT3.TEXT = Str$(Y2MAX(4))
    FORM9.TEXT4.TEXT = Str$(Y2MIN(4))
    FORM9.Show 1
    u4click
End Sub

Sub Check1_Click ()
    u4click
End Sub
** FORM FOR CHANGE GRAPH AXIS FORM 9

Sub Command2_Click()
Unload FORM9
End Sub

Sub Command1_Click()
Y1MAX(GRF) = Val(TEXT1.TEXT)
Y1MIN(GRF) = Val(TEXT2.TEXT)
Y2MAX(GRF) = Val(TEXT3.TEXT)
Y2MIN(GRF) = Val(TEXT4.TEXT)
Unload FORM9
End Sub

** FORM AND INSTRUCTIONS FOR OPENING FILES

* GETFILE FORM

Declarations for GETFILE.FRM

Const TEXTFLAG = 0
Const FILEFLAG = 1
Const DIRFLAG = 2

Dim SelectFlag As Integer

Sub File1_Click()
Text1.Text = File1.FileName
SelectFlag = FILEFLAG
End Sub

Sub File1_DblClick()

Text1.Text = File1.FileName
SelectFlag = FILEFLAG
FileSelected = TRUE
Command1_Click

End Sub

Sub Dir1_Change()
FillLabel1
File1.FileName = Dir1.Path + \\
\"\" + File1.Pattern
Drive1.Drive = Dir1.Path
Text1.Text = File1.Pattern
SelectFlag = DIRFLAG
End Sub

Sub Drive1_Change()
Dir1.Path = Drive1.Drive
Text1.Text = File1.Pattern
SelectFlag = DIRFLAG
End Sub

Sub Form_Load()
GetFile.Left = (Screen.Width - GetFile.Width) / 2
GetFile.Top = (Screen.Height - GetFile.Height) / 2

If FullFilePath <> "" Then
    Tmp$ = FullFilePath
    Do Until Right$(Tmp$, 1) = "\"
        Tmp$ = Left$(Tmp$, Len(Tmp$) - 1)
    Loop
    Tmp$ = Tmp$ + WILDCARD$
    File1.FileName = Tmp$
    Dir1.Path = File1.Path
End If

File1.Pattern = WILDCARD$
FillLabel1
Text1.Text = File1.Pattern
SelectFlag = DIRFLAG
FileSelected = FALSE
End Sub

Sub Command1_Click()
On Error GoTo ErrorTrap

If SelectFlag = TEXTFLAG Then
    File1.FileName = Text1.Text
    If FileSelected = TRUE Then
        On Error GoTo 0
        Unload GetFile
        Exit Sub
    End If
    Dir1.Path = File1.Path
ElseIf SelectFlag = DIRFLAG Then
    Dir1.Path = Dir1.List(Dir1.ListIndex)
    Dir1_Change
Else
    If Right$(Dir1.Path, 1) = "\" Then
        FullFilePath = Dir1.Path + Text1.Text
    Else
        FullFilePath = Dir1.Path + "\" + Text1.Text
    End If
    FileSelected = TRUE
Unload GetFile
'STop
Call fromfile
'STop
End If
Exit Sub
Sub Command2_Click ()
    Unload GetFile
End Sub

Sub Text1_Change ()
    SelectFlag = TEXTFLAG
End Sub

Sub Dir1_Click ()
    SelectFlag = DIRFLAG
End Sub

Sub FillLabel1 ()
    Label1.Caption = Dir1.Path
    If Label1.Width > 2055 Then
        a$ = Left$(Dir1.Path, 3)
        c$ = Mid$(Dir1.Path, 4)
        Do While InStr(c$, "\")
            c$ = Mid$(c$, InStr(c$, "\") + 1)
        Loop
        Label1.Caption = a$ + "\" + c$
    End If
End Sub

Sub Form_Resize ()
    Text1.SetFocus
End Sub

'** SUB ROUTINES NOT EMBEDDED IN FORMS

Sub OUTNAME ()
End Sub

Sub getdat ()

' SUBROUTINE FOR DATA
' 11-92 If B = 2 Then dio%(1) = 3400; dio%(2) = 600; dio%(4) = 100
  RENTIMEB = 5' used when no indication is given for rennet addition
  'If FILEDATA$ <> "ON" Then
  B = B + 1
  '  XL% = INP(768)
  '  XH% = INP(769)
CURVE

\[ DIO\%(1) = XL\% + 256 \times (XH\% \text{ And} \& HF) \]
\[ XL\% = \text{INP}(770) \]
\[ XH\% = \text{INP}(771) \]
\[ DIO\%(2) = XL\% + 256 \times (XH\% \text{ And} \& HF) \]
\[ XL\% = \text{INP}(772) \]
\[ XH\% = \text{INP}(773) \]
\[ DIO\%(3) = XL\% + 256 \times (XH\% \text{ And} \& HF) \]
\[ XL\% = \text{INP}(774) \]
\[ XH\% = \text{INP}(775) \]
\[ DIO\%(4) = XL\% + 256 \times (XH\% \text{ And} \& HF) \]

\[ \text{If} \quad B < 30 \quad \text{Then} \quad \text{INC} = 1 \]
\[ \text{If} \quad B > 30 \quad \text{Then} \quad \text{INC} = \text{INC} + 1 \]
\[ \text{If} \quad B > 40 \quad \text{Then} \quad \text{INC} = \text{INC} + 3 \]
\[ \text{If} \quad B > 50 \quad \text{Then} \quad \text{INC} = \text{INC} - 2 \]
\[ \text{If} \quad B > 60 \quad \text{And} \quad B < 70 \quad \text{Then} \quad \text{INC} = 1 \quad \text{'CREATE TEST COAG} \]
\[ \text{If} \quad B > 71 \quad \text{Then} \quad \text{INC} = \text{INC} + 1 \]
\[ \text{If} \quad B > 80 \quad \text{Then} \quad \text{INC} = \text{INC} + 2 \]
\[ \text{If} \quad B > 90 \quad \text{Then} \quad \text{INC} = \text{INC} - 2 \]
\[ \text{If} \quad B > 91 \quad \text{Then} \quad \text{INC} = -4 \]

\[ dio\%(1) = 3420 + \text{INC} \quad \text{***testing} \]
\[ dio\%(2) = 300 + DIO2\% / 98 \]
\[ dio\%(3) = 3000 \]
\[ dio\%(4) = 125 \]
\[ \text{TIMES}(B) = (B - 2) \times 5 / 60 \quad \text{'minutes running} \]

If \( dio\%(1) < 20 \) Then Return

' \text{FIX ABOVE LINE FOR ERROR CORRECTION} \]
\[ N = 2 \]

'DATA COLLECTION
\[ \text{PH1} = dio\%(2) \]
\[ \text{PH}(B) = 7 - ((\text{PH1} - 24) / 300) \]

'section GETs RTD DATA FROM RTD probe

'RTD SUB
\[ E = 2 \]
\[ RX = (dio\%(4) \times 7.700001E - 02) + 100 \]

'\text{--- EVALUATE POLYNOMIAL TO DETERMINE TEMPERATURE} \]
\[ \text{RCOE}(1) = -242.8382 \]
\[ \text{RCOE}(2) = 2.275128 \]
\[ \text{RCOE}(3) = 1.879644E - 03 \]
\[ \text{RCOE}(4) = -4.554426E - 06 \]
\[ \text{RCOE}(5) = 1.132138E - 08 \]
\[ \text{RCOE}(6) = -8.142306E - 12 \]
\[ \text{DEGC}(E) = \text{RCOE}(1) \]
For CNT% = 5 To 1 Step -1
DEGC(E) = DEGC(E) + RCOEF(CNT% + 1) * RX^CNT%
Next CNT%
RTD(B) = DEGC(E)

Proprietary equations deleted!!!!!!!

If B > 15 Then
HEATRATE = (RTD(B - 1) - RTD(B - 4)) / (TIMES(B - 1) - TIMES(B - 4))
End If
If B = 1 Then Return
'HP SMOOTHING AND DTS RECALCULATION
If B = 4 Then
For N = 1 To 3
TH(N) = TH(4)
RTD(N) = RTD(4)
Next N
End If
Write #2, TIMES(B), RTD(B), PH(B), DTS(B), TH(B)"
GoSub DTSRECALC

COUNTA = COUNTA + 1: COUNTB = COUNTB + 1
CHECKDT = 0
If B = 1 Then THBEFORE = TH(B)
If TH(B) > THBEFORE Then POSDT = -1
If TH(B) < THBEFORE Then POSDT = 1
If Abs(TH(B) - THBEFORE) > .1 Then FLAGJUMPTH = 1
If TH(B) <> THBEFORE Then GoSub HPAVG
If CHECKDT <> 1 Then TH(B) = TH(B) + (.0425825 * POSDT)

CHECKRT = 0
If B < 4 Then RTBEFORE = RTD(B)
If RTD(B) > RTBEFORE Then POSRT = -1
If RTD(B) < RTBEFORE Then POSRT = 1
If Abs(RTD(B) - RTBEFORE) > .4 Then FLAGJUMPRRT = 1
If RTD(B) <> RTBEFORE Then GoSub RTDAVG
If CHECKRT <> 1 Then RTD(B) = RTD(B) + (.09905 * POSRT)
If B > 3 Then MAXMIN2 'WAS MAXMIN 11-14-92

FORM3.PICTURE1.DRAWWIDTH = 1
FORM3.PICTURE2.DRAWWIDTH = 1
FORM3.PICTURE3.DRAWWIDTH = 1
FORM3.PICTURE4.DRAWWIDTH = 1

FORM3.PICTURE1.Cls
FORM3.PICTURE2.Cls
FORM3.PICTURE3.Cls
FORM3.PICTURE4.Cls
For A = 1250 To 3750 Step 1250 ' PUTS GRID LINES IN
  FORM3.PICTURE1.Line (0, A)-(5000, A)
  FORM3.PICTURE2.Line (0, A)-(5000, A)
  FORM3.PICTURE3.Line (0, A)-(5000, A)
  FORM3.PICTURE4.Line (0, A)-(5000, A)
Next A
  FORM3.PICTURE1.DRAWWIDTH = 4
  FORM3.PICTURE2.DRAWWIDTH = 4
  FORM3.PICTURE3.DRAWWIDTH = 4
  FORM3.PICTURE4.DRAWWIDTH = 4

FORM3.LABEL5(2).CAPTION = Format$(PH(B), "0.00")
FORM3.LABEL5(3).CAPTION = "PH"
FORM3.LABEL5(0).CAPTION = Format$(ylmax(1), "0.00")
FORM3.LABEL5(1).CAPTION = Format$(ylmin(1), "0.00")
dif1 = ylmax(1) - ylmin(1); dif2 = PH(B) - ylmin(1)

If PH(B) > ylmin(1) And PH(B) < ylmax(1) Then
  FORM3.PICTURE1.Line (0, 5000 - (dif2 / dif1 * 5000))-(5000, 5000 - (dif2 /
  dif1 * 5000))
End If

FORM3.LABEL6(2).CAPTION = Format$(RTD(B), "0.00")
FORM3.LABEL6(3).CAPTION = "SAMP TEMP"
FORM3.LABEL6(0).CAPTION = Format$(y2max(1), "0.00")
FORM3.LABEL6(1).CAPTION = Format$(y2min(1), "0.00")
dif1 = y2max(1) - y2min(1); dif2 = RTD(B) - y2min(1)

If RTD(B) > y2min(1) And RTD(B) < y2max(1) Then
  FORM3.PICTURE2.Line (0, 5000 - (dif2 / dif1 * 5000))-(5000, 5000 - (dif2 /
  dif1 * 5000))
End If

FORM3.LABEL7(2).CAPTION = Format$(DTS(B), "0.00")
FORM3.LABEL7(3).CAPTION = "HOT WIRE"
FORM3.LABEL7(0).CAPTION = Format$(y1max(2), "0.00")
FORM3.LABEL7(1).CAPTION = Format$(y1min(2), "0.00")
dif1 = y1max(2) - y1min(2); dif2 = DTS(B) - y1min(2)

If DTS(B) > y1min(2) And DTS(B) < y1max(2) Then
  FORM3.PICTURE3.Line (0, 5000 - (dif2 / dif1 * 5000))-(5000, 5000 - (dif2 /
  dif1 * 5000))
End If

FORM3.LABEL8(2).CAPTION = Format$(DER1(B), "0.00")
FORM3.LABEL8(3).CAPTION = "1ST DERIV"
FORM3.LABEL8(0).CAPTION = Format$(y2max(2), "0.00")
FORM3.LABEL8(1).CAPTION = Format$(y2min(2), "0.00")
dif1 = y2max(2) - y2min(2)
dif2 = DER1(B) - y2min(2)

If DER1(B) > y2min(2) And DER1(B) < y2max(2) Then
    FORM3.PICTURE4.Line (0, 5000 - (dif2 / dif1 * 5000))-(5000, 5000 - (dif2 / dif1 * 5000))
End If
' Stop

FORM3.LABEL4(0).CAPTION = "MINUTES RUNNING = ":
FORM3.LABEL4(1).CAPTION = Format$(COUNTDAT(1) / 12, "0.0")

If FORM5STAT$ = "ON" Then
    FORM5.PICTURE1(0).Cls: FORM5.PICTURE1(0).Print Format$(PH(B), "0.00")
    FORM5.PICTURE1(1).Cls: FORM5.PICTURE1(1).Print Format$(RTD(B), "0.00")
    FORM5.PICTURE1(2).Cls: FORM5.PICTURE1(2).Print Format$(DTS(B), "0.00")
    If B > 10 Then
        FORM5.PICTURE1(3).Cls: FORM5.PICTURE1(3).Print Format$(SLOPETESTT(B - 7), "0.00")
    End If
    FORM5.PICTURE1(4).Cls: FORM5.PICTURE1(4).Print Format$(TIMES(B), "0.00")

    FORM5.PICTURE2(0).Cls: FORM5.PICTURE2(0).Print Format$(TIMES(RENTIMEB), "0.00”), Format$(PH(RENTIMEB), "0.00”), Format$(RTD(RENTIMEB), "0.00")
    FORM5.PICTURE2(1).Cls: FORM5.PICTURE2(1).Print Format$(TIMES(SLOPEMAXB), "0.00”), Format$(PH(SLOPEMAXB), "0.00”), Format$(RTD(SLOPEMAXB), "0.00")
    FORM5.PICTURE2(2).Cls: FORM5.PICTURE2(2).Print Format$(TIMES(TIMECUTB), "0.00”), Format$(PH(TIMECUTB), "0.00”), Format$(RTD(TIMECUTB), "0.00")
    FORM5.PICTURE2(3).Cls: FORM5.PICTURE2(3).Print Format$(TIMES(TIMEHEALB), "0.00”), Format$(PH(TIMEHEALB), "0.00”), Format$(RTD(TIMEHEALB), "0.00")
    FORM5.PICTURE2(4).Cls: FORM5.PICTURE2(4).Print Format$(ESTCUT, "0.0")
End If
Exit Sub '********** IS THIS OK 11-17-92
DTSRECALC:
' ---------- SUB RECALCULATES DTS
NEWCOUNTA = COUNTA
NUMBER = B - 1
'COUNTTB = COUNTB3 + COUNTB2 + COUNTB"GOES WITH DTS RECALC
If NEWCOUNTA > NEWCOUNTTB Then HIGHNUMBER = NEWCOUNTA
If NEWCOUNTB > NEWCOUNTA Then HIGHNUMBER = NEWCOUNTB
LOWNUMBER = B - (HIGHNUMBER + 20)
If LOWNUMBER < 4 Then LOWNUMBER = 4
'LOWNUMBER AND HIGHNUMBER PREVENT RECALCULATION OF ALL DTS VALUES

For N = LOWNUMBER To NUMBER
TS = RTD(N)

Proprietary equations hidden!!!!

Next N
Return

HPAVG:
' ---------- SUB AVERAGES HOT PROBE TEMPERATURE
POSDTA = 1: CHECKDT = 1
THBEFORE = TH(B)
TH(B) = TH(B) + (.0425825 * POSDT)
COUNT = COUNTA - 1
U = B - COUNT
If FLAGJUMPTH = 1 Then
WIRETEMP = TH(B - 1) + (2 *.0425825 * POSDT * (-1))
Else WIRETEMP = TH(B)
End If
DIFA = (WIRETEMP - TH(U - 1)) / COUNTA
If FLAGJUMPTH = 1 Then COUNTA = COUNTA - 1

For N = 1 To COUNTA
TH(U) = TH(U - 1) + DIFA
U = U + 1
Next N
COUNTA = 0
FLAGJUMPTH = 0
Return

RTDAV:
' ---------- SUB AVERAGES RTD TEMPERATURE
POSRITA = 1: CHECKRT = 1
RTBEFORE = RTD(B)
If PCHK4 + PCHK3 = 0 Then COUNTB2 = COUNTB2 + COUNTB3'
PCHK4 = PCHK3 ' CHECKS PATTERN FOR A FALSE RISE AND
PCHK3 = PCHK2 ' FALL IN THE TEMPERATURE
PCHK2 = PCHK1
PCHK1 = POSRT
COUNTB3 = COUNTB2
COUNTB2 = COUNTB1
COUNTB1 = COUNTB
If Abs(PCHK1 + PCHK2) = 2 Then
  If PCHK4 + PCHK3 = 0 And PCHK3 + PCHK2 = 0 Then
    NEWCOUNTB = COUNTB3 + COUNTB2 + COUNTB1
  Else NEWCOUNTB = COUNTB
End If
Else NEWCOUNTB = COUNTB
End If

If NEWCOUNTB > B Then NEWCOUNTB = B - 1
  RTD(B) = RTD(B) + (.09905 * POSRT)
  COUNT = NEWCOUNTB - 1
  U = B - COUNT
  If FLAGJUMPRT = 1 Then
    VATTEMP = RTD(B - 1) + (4 * .09905 * POSRT * (-1))"
  Else VATTEMP = RTD(B)
  End If
  DIFB = (VATTEMP - RTD(U - 1)) / NEWCOUNTB
  If FLAGJUMPRT = 1 Then NEWCOUNTB = NEWCOUNTB - 1
  For N = 1 To NEWCOUNTB
    RTD(U) = RTD(U - 1) + DIFB
    U = U + 1
  Next N
  COUNTB = 0
  FLAGJUMPRT = 0
  Return

End Sub

Sub PRIM ()
End Sub

Sub main ()

TESTDATA$ = FILENAME$(c)
ACTION$ = ""
"Open "HPMAIN.SET" For "input As #1
" Input #1, SHUTOFF$, OUTINTFILE$
  MTM = 50: BET = 5
"Close #1

RUNCOUNT = RUNCOUNT + 1
'PROGRAM SET SO IT WILL RUN THROUGH 6 TIMES IF NOT STOPPED
ABT 5 HOURS
A$ = ""
"cls

If OVERTIME = 0 Then
  CUTCHECK$ = "NO": HEALCHECK$ = "NO": COAGCHECK$ = "NO"
  MAXSTUFFSTARTED = 0: WRITECOUNT = 0: WRITECOUNT1$ = ""
  RENTIME = 0: OVERTIME = 0

***************CONSTANTS FOR PROBE 1205

proprietary equations hidden!!!

End If

RTDFLAG = 0

'IF TIME OVER THEN SKIP
While dio%(1) < 1000
  getdat
  "Print "CHECKING FOR HOT.PROBE CONNECTION"
  B = B - 1
Wend
B = 3
For N = 1 To 100
  "Print "CHECKING FOR HOT.PROBE CONNECTION"
Next N

getdat
'IF FILEDATA$ <> "ON" THEN TIMER ON
'ON TIMER(5) GOSUB GETDATA

PRIM
'FORMX TIMER1=FALSE
c = 1
FILENAM$ = FILENAME$(c) + WRITECOUNT1$ + ".CSV"
Open FILENAM$ For Output As #1
' PRINT #1," TIME TEMP PH
PRINT #1,"RENNET:";TIMES(RENTIMEB),RTD(RENTIMEB),PH(RENTIMEB)
' PRINT #1,"COAG: ";TIMEMAX,TIMEMAXTEMP,TIMEMAXPH
' PRINT #1,"CUT: ";TIMECUT,TEMPCUTTEMP,TIMECUTPH
' PRINT #1,"HEAL: ";TIMEHEAL,TIMEHEALTEMP,TIMEHEALPH
' PRINT #1,"MAX TEMP.";TEMPMAX
U = 1
Print #1, FILENAME$(c), DESCRIP$
For U = 3 To B - 2 'STEP 5
  Write #1, TIMES(U), RTD(U), PH(U), DTS(U), TH(U)
Next U
Close #1
"VIEW PRINT

FILENAME$ = FILENAME$(c) + WRITECOUNT1$ + ".MAX"

If COAGCHECK$ = "YES" Then

Open FILENAME$ For Output As #1
SMB = SLOPEMAXB - 20
If SLOPEMAXB - 20 < 0 Then SMB = 1
SMC = SLOPEMAXB + 40
If SLOPEMAXB + 40 < B Then SMC = B - 3
For U = SMB To SMC
    Write #1, TIMES(U), RTD(U), PH(U), DTS(U), TH(U)
Next U
Print #1, FILENAME$, DESCRIP$
Close #1

End If ' END IF
WRITECOUNT = WRITECOUNT + 1
WRITECOUNT1$ = LTrim$(Str$(WRITECOUNT))

" GoSub ints

"VIEW PRINT
OVERTIME = 1

SLOPEMAX = 0
SLOPEMIN = 0: DTSMAX = 0
TEMPMAX = 0: SLOPEMAXB = 0

If SHUTOFF$ = "MANUEL" And STOPFLAG$ <> "Y" And RUNCOUNT < 7 Then
    B = 2
    main
End If

"INPUT "DO YOU WANT TO RUN ANOTHER"; ANOTHER$
"If ANOTHER$ = "Y" Or ANOTHER$ = "y" Then GoSub STARTSUB
Close #2
End Sub
Sub fromfile ()

End Sub
Sub fromfile ()
RENTIME = 1
DEMO$ = "": NEWDATA$ = ""
'Stop
'On Error GoTo WHATHAPPENED

Open fullfilepath For Input As #1
FOR v = 1 TO 8
Line Input #1, nothing$
Line Input #1, nothing$

NEXT v
COUNTC = 1
DATAINGRAF = 0

FORM3.CURRENTX = 1000: FORM3.CURRENTY = 3000
FORM3.Print "PLEASE WAIT, WORKING!"

'*********
While Not EOF(1)
  Input #1, fftime, FFRD, fFPHN, fFDTS, fFTH
  FTIMES(COUNTC) = fftime: FRTD(COUNTC) = FFRD:
  FPh(COUNTC) = fFPHN: FDTs(COUNTC) = fFDTS
  FORM1.Cls
  FORM1.Print "TIME = "; fftime

  TESTCOUNT = TESTCOUNT + 1
  MTM = FTIMES(COUNTC)
  interval = FTIMES(COUNTC) - FTIMES(COUNTC - 1)

  If MTM > 29 Then COUNTC = COUNTC - 1
  '******JUST FOR TEST OF TOO MANY DATA POINTS
  fXMAX = MTM

  COUNTC = COUNTC + 1

Wend
Close #1

RENTIMEBF = 5TESTING
FMAXCHECK = 2.8
FCUTCHECK$ = "NO": FHEALCHECK$ = "NO"

For XX = 10 To COUNTC - 10
'F DENOTES DATA FROM FILE

If FMAXSTUFFSTARTED <> 1 Then
  FSLOPEMAX = 0
  FSLOPEMIN = 0; FDTSMAX = 0;
  FTEMPMAX = 0: FMAXSTUFFSTARTED = 1
End If

SLOPETEST1 = (FDTS(XX + 3) + FDTS(XX + 4) + FDTS(XX + 5) +
  FDTS(XX + 6)) / 3
SLOPETEST2 = (FDTS(XX - 3) + FDTS(XX - 4) + FDTS(XX - 5) +
  FDTS(XX - 6)) / 3
SLOPETESTT(XX) = SLOPETEST1 - SLOPETEST2
SLOPETESTF = SLOPETESTT(XX)
'SLOPETEST = FDTS(N) - FDTS(N - 2)

If CUTCHECK$ <> "YES" Then
  If FSLOPEMAX < SLOPETESTF Then FTIMEMAX = FTIMES(XX)
  If FSLOPEMAX < SLOPETESTF And XX > RENTIMEBF Then
    FSLOPEMAXB = XX
  End If
  If FSLOPEMAX < SLOPETESTF Then FSLOPEMAX = SLOPETESTF
  If FDTSMAX < FDTS(XX) Then FDTSMAX = FDTS(XX)
End If

If FDTSMAX > FMAXCHECK And FCUTCHECK$ = "NO" Then
  If (FDTS(XX) - FDTS(XX - 1)) < -.2 Then FTIMECUTB = XX ': Stop
  If (FDTS(XX) - FDTS(XX - 1)) < -.2 And FDTSMAX > FMAXCHECK
  Then
    FCUTCHECK$ = "YES"
  End If
End If
End If

If FCUTCHECK$ = "YES" And FHEALCHECK$ <> "YES" Then
  If FTIMES(XX) > FTIMES(FTIMECUTB) + 5 Then
    If (FDTS(XX) - FDTS(XX - 1)) < -.2 Then FTIMEHEALB = XX
    If (FDTS(XX) - FDTS(XX - 1)) < -.2 Then FHEALCHECK$ =
  "YES"
  End If
End If

If FRTD(XX) > FTEMPMAX Then FTEMPMAX = FRTD(XX)
  FTEMPMAXTEMP = FRTD(FSLOPEMAXB): FTEMPMAXPH =
  FPPh(FSLOPEMAXB)
  FTIMECUT = FTIMES(FTIMECUTB): FTIMECUTTEMP =
  FRTD(FTIMECUTB)
  FTIMECUTPH = FPPh(FTIMECUTB)
  FTIMEHEAL = FTIMES(FTIMEHEALB): FTIMEHEALTEMP =
  FRTD(FTIMEHEALB)
  FTIMEHEALPH = FPPh(FTIMEHEALB)
FHEATRATE = (FRTD(XX) - FRTD(XX - 4)) / (FTIMES(XX) - FTIMES(XX - 4))

' Stop
FTIMEMAX = (FTIMES(FSLOPEMAXB))
' FORM7.Print
Next XX
FRATIO = (TIMECUT - FTIMEMAX) / FTIMEMAX
FHEALTOTAL = FTIMEHEAL - TIMECUT

' End If STOP
BoxNormForm FORM3, 1000, 2999, 3000, 500, GRAY
FORM3.Show
UT3GRF.Show
UT4GRF.Show
U3CLICK
U4CLICK
FORM7.Show
FORM7.Cls
FORM7.CAPTION = "COAGULATION DATA"
FORM7.PICTURE1(0).Cls: FORM7.PICTURE1(0).Print
Format$(FTIMEMAX, " 0.00")
FORM7.PICTURE1(1).Cls: FORM7.PICTURE1(1).Print
Format$(FTIMEMAXPH, " 0.00")
FORM7.PICTURE1(2).Cls: FORM7.PICTURE1(2).Print
Format$(FTIMEMAXTEMP, " 0.00")
FORM7.PICTURE1(3).Cls: FORM7.PICTURE1(3).Print
Format$(FSLOPEMAX, " 0.00")
Format$(TIMECUT, " 0.00")
FORM7.PICTURE1(5).Cls: FORM7.PICTURE1(5).Print
Format$(TIMECUTPH, " 0.00")
Format$(TIMECUTTEMP, " 0.00")
FORM7.PICTURE1(7).Cls: FORM7.PICTURE1(7).Print
Format$(FRATIO, " 0.00")
FORM7.PICTURE1(8).Cls: FORM7.PICTURE1(8).Print
Format$(FTIMEHEAL, " 0.00")
FORM7.PICTURE1(9).Cls: FORM7.PICTURE1(9).Print
Format$(FTIMEHEALPH, " 0.00")
FORM7.PICTURE1(10).Cls: FORM7.PICTURE1(10).Print
Format$(FTIMEHEALTEMP, " 0.00")
Format$(FHEALTOTAL, " 0.00")
BOXFORM FORM7, 20, 20, 5120, 3700, GRAY
FORM7.CURRENTX = 0: FORM7.CURRENTY = 0
FORM7.Print Tab(22); fullfilepath
DBBOXFORM FORM7, 440, 560, 800, 325, GRAY
End Sub

Sub MAXMIN2()

N = B - 7
If N > RENTIMEB + 7 Or RENTIME <> 0 Then
tempdif = RTD(B) - FRTD(B)
If MAXSTUFFSTARTED <> 1 Then
   FINISH = B - 1: SLOPEMAX = 0:
   SLOPEMIN = 0: DTSMAX = 0:
   TEMPAX = 0: MAXSTUFFSTARTED = 1
End If
SLOPETEST1 = (DTS(N + 4) + DTS(N + 5) + DTS(N + 6)) / 3
SLOPETEST2 = (DTS(N - 4) + DTS(N - 5) + DTS(N - 6)) / 3
SLOPETESTT(N) = SLOPETEST1 - SLOPETEST2
SLOPETEST = SLOPETESTT(N)
'SLOPETEST = DTS(N) - DTS(N - 2)
slopetestdif = slopetest - slopetestf 'DIFFERENCE FROM NORMAL
If CUTCHECKS <> "YES" Then
   If SLOPEMAX < SLOPETEST Then TIMEMAX = TIMES(N):
   SLOPEMAXB = N
End If

End Sub
'If SLOPEMAX < SLOPETEST And N > RENTIMEB Then
SLOPEMAXB = N
If SLOPEMAX < SLOPETEST Then SLOPEMAX = SLOPETEST
If DTSMAX < DTS(N) Then DTSMAX = DTS(N)
dtsmaxdif = DTSMAX - DTSMAX
timedif = TIMEMAX - FTIMEMAX
End If

If DTSMAX > MAXCHECK And CUTCHECK$ <> "YES" Then
If (DTS(N) - DTS(N - 1)) < -.2 Then TIMECUTB = N
If (DTS(N) - DTS(N - 1)) < -.2 And DTSMAX > MAXCHECK Then
CUTCHECK$ = "YES"
End If
End If
If CUTCHECK$ = "YES" And HEALCHECK$ <> "YES" Then
If TIMES(N) > TIMES(TIMECUTB) + 1 Then *** USE BIGGER
NUMBER FOR REAL STUFF @6
If (DTS(N) - DTS(N - 1)) < -.2 Then TIMEHEALB = N
If (DTS(N) - DTS(N - 1)) < -.2 Then HEALCHECK$ = "YES"
End If
End If

If RTD(N) > TEMPMAX Then TEMPMAX = RTD(N)
TIMEMAXTEMP = RTD(SLOPEMAXB): TIMEMAXPH =
PH(SLOPEMAXB)
TIMECUT = TIMES(TIMECUTB): TIMECUTTEMP = RTD(TIMECUTB)
TIMECUTPH = PH(TIMECUTB)
TIMEHEAL = TIMES(TIMEHEALB): TIMEHEALTEMP =
RTD(TIMEHEALB)
TIMEHEALPH = PH(TIMEHEALB)
HEATRATE = (RTD(N) - RTD(N - 4)) / (TIMES(N) - TIMES(N - 4))
If SLOPEMAXB > 1 Then
ESTCUT = (TIMES(SLOPEMAXB) - TIMES(RENTIMEB)) * 2 +
TIMES(RENTIMEB)
ESTCUT2 = -265.9*(SLOPEMAX) +57.62
'CUT ESTIMATE BASED ON 1:1 REN-TO-CLOT...CLOT-TO-CUT
End If
End If
End Sub

Sub WRITENUMBERS ()
FORM7.Cls
FORM7.Print Format$(FTIMEMAX, "0.00")
FORM7.PICTURE1(0).Cls
FORM7.PICTURE1(0).Print Format$(FTIMEMAX, "0.00")
FORM7.PICTURE1(1).Cls: FORM7.PICTURE1(1).Print
Format$(TIMEMAXPH, "0.00")
FORM7.PICTURE1(2).Cls: FORM7.PICTURE1(2).Print
Format$(FTIMEMAXTEMP, "0.00")
FORM7.PICTURE1(3).Cls:
FORM7.PICTURE1(3).Cls
FORM7.PICTURE1(3).Print Format$(FSLOPEMAX, "0.00")

End Sub

'** SUBROUTINES I WROTE TO DRAW 3D BOXES ON SCREENS IN DIFFERENT ** FORMS
Sub BoxControl (CONTROLX As Control, X As Integer, Y As Integer, DX As Integer, DY As Integer, clr)
  CONTROLX.Line (X, Y)-(DX + X, DY + Y), clr, BF
  CONTROLX.Line (X, Y)-(DX + X, DY + Y), BLACK, B
  CONTROLX.Line (X, Y)-(X, DY + Y), WHITE
  CONTROLX.Line (X, Y)-(DX + X, Y), WHITE
'CONTROLX As Control, FORMX As Form,
End Sub

Sub BOXFORM (FORMX As Form, X As Integer, Y As Integer, DX As Integer, DY As Integer, cir)
  FORMX.Line (X, Y)-(DX + X, DY + Y), clr, BF
  FORMX.Line (X, Y)-(DX + X, DY + Y), BLACK, B
  FORMX.Line (X, Y)-(X, DY + Y), WHITE
  FORMX.Line (X, Y)-(DX + X, Y), WHITE
'CONTROLX As Control, FORMX As Form,
End Sub

Sub setmark (FORMX As Form, xset, yset, clr)
  'formx.Line (xset, yset - 75)-(xset + 75, yset + 100), white, BF
  'formx.Line (xset, yset)-(xset - 75, yset - 75), clr
  'formx.Line (xset, yset)-(xset - 75, yset + 75), clr
  'formx.Line (xset - 75, yset - 75)-(xset - 75, yset + 75), clr
End Sub

Sub dBBoxControl (CONTROLX As Control, X As Integer, Y As Integer, DX As Integer, DY As Integer, clr)
  If clr = WHITE Then clr2 = gray Else clr2 = WHITE
  CONTROLX.Line (X, Y)-(DX + X, DY + Y), clr, BF
  CONTROLX.Line (X, Y)-(DX + X, DY + Y), BLACK, B
  CONTROLX.Line (X, Y)-(X, DY + Y), clr2
  CONTROLX.Line (X, Y)-(DX + X, Y), clr2
  CONTROLX.Line (X + 25, Y + 25)-(DX + X - 25, DY + Y - 25), clr2, B
  CONTROLX.Line (X + 25, Y + 25)-(X + 25, DY + Y - 25), BLACK
CONTROLX.Line (X + 25, Y + 25)-(DX + X - 25, Y + 25), BLACK

End Sub

Sub BoxNormControl (CONTROLX As Control, X As Integer, Y As Integer, DX As Integer, DY As Integer, clr)
    CONTROLX.Line (X, Y)-(DX + X, DY + Y), clr, BF
End Sub

Sub BoxNormForm (FORMX As Form, X As Integer, Y As Integer, DX As Integer, DY As Integer, clr)
    FORMX.Line (X, Y)-(DX + X, DY + Y), clr, BF
End Sub

Sub DBOXFORM (FORMX As Form, X As Integer, Y As Integer, DX As Integer, DY As Integer, clr)
    FORMX.Line (X, Y)-(DX + X, DY + Y), clr, BF
    FORMX.Line (X, Y)-(DX + X, DY + Y), WHITE, B
    FORMX.Line (X, Y)-(X, DY + Y), BLACK
    FORMX.Line (X, Y)-(DX + X, Y), BLACK
End Sub

Sub BOXINFORM (FORMX As Form, X As Integer, Y As Integer, DX As Integer, DY As Integer, cir)
    FORMX.Line (X, Y)-(DX + X, DY + Y), cir, BF
    FORMX.Line (X, Y)-(DX + X, DY + Y), WHITE, B
    FORMX.Line (X, Y)-(X, DY + Y), BLACK
    FORMX.Line (X, Y)-(DX + X, Y), BLACK
End Sub

Sub BOXFORM_TEXT (FORMX As Form, X As Integer, Y As Integer, DX As Integer, DY As Integer, clr, TEXT$)
    FORMX.Line (X, Y)-(DX + X, DY + Y), clr, BF
    FORMX.Line (X, Y)-(DX + X, DY + Y), WHITE, B
    FORMX.Line (X, Y)-(X, DY + Y), BLACK
    FORMX.Line (X, Y)-(DX + X, Y), BLACK
    FORMX.CURRENTX = X + (DX * .1): FORMX.CURRENTY = Y + (DY / 3)
    FORMX.Print TEXT$
End Sub
Sub BOXinCONTROL (CONTROLX As Control, X As Integer, Y As Integer, 
DX As Integer, DY As Integer, clr)
CONTROLX.Line (X, Y)-(DX + X, DY + Y), clr, BF
CONTROLX.Line (X, Y)-(DX + X, DY + Y), WHITE, B
CONTROLX.Line (X, Y)-(X, DY + Y), BLACK
CONTROLX.Line (X, Y)-(DX + X, Y), BLACK
End Sub
Appendix B

Zymate® Robot Control Program
Zymate® Robot Control Program

PROGRAM FOR ROBOT CONTROL AND AUTOMATION

EASYLAB PROGRAM: HOT.PROBE.3

J 0
SAMPLE.NUM = 0
INPUT TOTAL.SAMPLES
INPUT CULTURE.VOLUME
INPUT RENNENET.VOLUME

100 GET.CULTURE
PLACE. IN.WARMER
STIR.CULTURE
REMOVE. FLEAKER.FROM.STIRPLATE
PUT.CULTURE.IN.ICE.BATH
REMOVE.CAP
GET.MILK.SAMPLE.M
PLACE.IN.WARMER
STIRPLATE = 200
PUMPON
ADD.CULTURE.TO.MILK
GET.PROBE
PLACE.PROBE.IN.SAMPLE
H.PROBE.ON
PARK.HAND.3
CHECK.INCUBATION.TEMPERATURE
PUMP.OFF
SET TIMER 2 240 SECONDS
WAIT FOR TIMER 2
ADD.RENNENET
GET.HAND.3
STIRPLATE = 0
WAIT.FOR.DATA.COLLECTION1
PUMPON
STIRPLATE = 200
CHECK.COOK.TEMPERATURE
PUMP.OFF
STIRPLATE = 100
WAIT.FOR.COOK1
STIRPLATE.0
HPROBE.OFF
REMOVE.PROBE.FROM.SAMPLE
REMOVE.FLEAKER.FROM.STIRPLATE
RETURN.FLEAKER.TO.BATH
IF SAMPLE.NUM < TOTAL.SAMPLES THEN 100
PARK.HAND.3
EASYLAB PROGRAM: GET.CULTURE

GET.HAND.3
CLEAR.TO.ICE.BATH
WRIST = 186
SET.ABS.OVER.CULTURE
GRIP = 200
VERTICAL = 12.5
VERTICAL = 10.5
GRIP = 100
GRIP = 30
SET.ABS.OVER.CULTURE
CLEAR.TO.ICE.BATH

EASYLAB PROGRAM: PLACE.IN.WARMER
CLEAR.TO.WARMER
OVER.WARMER
SET.ABS.OVER.WARMER
VERTICAL = 20.7
VERTICAL = VERTICAL-3.2
GRIP = 200
VERTICAL = 21.7
CLEAR.TO.WARMER

EASYLAB PROGRAM: STIR.CULTURE

STIRPLATE = 200
SET TIMER 1 60 SECONDS
WAIT FOR TIMER 1
STIRPLATE 0

EASYLAB PROGRAM: REMOVE.FLEAKER.FROM.STIRPLATE

VERTICAL = 35
CLEAR.TO.WARMER
WRIST = 186
GRIP = 200
OVER.WARMER
SET.ABS.OVER.WARMER
VERTICAL = 20.7
VERTICAL = VERTICAL-3.2
GRIP = 30
SET.ABS.OVER.WARMER
VERTICAL = VERTICAL+15
CLEAR.TO.WARMER
EASYLAB PROGRAM: PUT.CULTURE.IN.ICE.BATH

CLEAR.TO.ICE.BATH
SET.ABS.OVER.CULTURE
VERTICAL = 12.5
VERTICAL = 10.7
GRIP = 200
SET.ABS.OVER.CULTURE
CLEAR.TO.ICE.BATH

EASYLAB PROGRAM: REMOVE.CAP

CLEAR.TO.ICE.BATH
WRIST = 186
GRIP = 200
IF SAMPLE.NUM > 0 THEN 10
SAMPLE.NUM = 0
10 SAMPLE.NUM = SAMPLE.NUM + 1
ICE.BATH.RACK.INDEX = SAMPLE.NUM
ICE.BATH.RACK
SET.ABS ICE.BATH.RACK
VERTICAL = VERTICAL - 7
VERTICAL = 13.5
GRIP = 120
ICE.BATH.RACK
CLEAR.TO.ICE.BATH
CLEAR.TO.CAP.WASTE
VERTICAL = 13
GRIP = 200
CLEAR.TO.CAP.WASTE
CLEAR.TO.ICE.BATH

EASYLAB PROGRAM: GET.MILK.SAMPLE.M

GET.HAND.3
CLEAR.TO.ICE.BATH
TRANS.ON
WRIST = 186
GRIP = 200
ICE.BATH.RACK.INDEX SAMPLE.NUM
ICE.BATH.RACK
SET.ABS ICE.BATH.RACK
VERTICAL = 13
VERTICAL = 10.7
GRIP = 100
GRIP = 30
VERTICAL = 24
CLEAR.TO.ICE.BATH
TRANS.OFF

EASYLAB PROGRAM: ADD.CULTURE.TO.MILK

IF HAND.ID = 0 THEN 5
IF HAND.ID = 3 THEN 3
3 PARK.HAND.3
GET.HAND.4
5 CLEAR.TO.ICE.BATH
GET.HAND.4
J = J+1
GET.1ML.TIP
CLEAR.TO.ICE.BATH
TIP.OVER.CULTURE
ADD.VOLUME=CULTURE.VOLUME
15 IF ADD.VOLUME>1 THEN 20
IF ADD.VOLUME<1 THEN 30
20 ASPIRATE.VOL.1
IF ADD.VOLUME>1 THEN 40
30 ASPIRATE.VOL.=ADD.VOLUME
40 SYRINGE = SYRINGE+5
TIP.OVER.=CULTURE
VERTICAL=VERTICAL-7
SYRINGE = SYRINGE+ASPIRATE.VOL.*200/2.5
TIP.OVER.CULTURE
SYRINGE = SYRINGE+5
CLEAR.TO.WARMER
TIP.OVER.SAMPLE
VERTICAL = VERTICAL-5
DISPENSE.SYRINGE
ADD.VOLUME=ADD.VOLUME-1
VERTICAL = VERTICAL+7
REACH = 0
IF ADD.VOLUME>0 THEN 15
CLEAR.TO.ICE.BATH
REMOVE.1ML.TIP
PARK.HAND.4
CLEAR.TO.ICE.BATH

EASYLAB PROGRAM: GET.PROBE

GET.HAND.3
CLEAR.TO.GET.PROBE
WRIST = 0
GRIP = 200
BEFORE.PROBE
IN.TO.Grab.PROBE
GRIP = 7
OVER.PROBE
RINSE.PROBE.ONSE
CLEAR.TO.PROBE

EASYLAB PROGRAM: PLACE.PROBE.IN.SAMPLE
CLEAR.TO.WARMER
PROBE.OVER.SAMPLE
PROBE.DOWN.TO.SAMPLE
GRIP = 30
GRIP = 200
REACH = 0

EASYLAB PROGRAM: PARK.HAND.3
COLL.OFF
WRIST 0
SET.ABS.AT.HAND.3
CLEAR.HAND.3
ABOVE.HAND.3
HAND.ID = 0
SET TIMER 4 1 SECONDS
WAIT FOR TIMER 4
BEFORE.HAND.3
AT.HAND.3

EASYLAB PROGRAM: CHECK.INCUBATION.TEMPERATURE
10  FLAGTEST 1
    IF RTDFLAG < 300 THEN GOTO 10

EASYLAB PROGRAM: ADD.RENNET
GET.HAND.4
J = J+1
GET.1ML.TIP
CLEAR.TO.ICE.BATH
OVER.RENNET
SYRINGE = SYRINGE+5
VERTICAL = VERTICAL-5
ASPIRATE.VOL = RENNET.VOL
SYRINGE = SYRINGE + ASPIRATE.VOL*200/2.5
VERTICAL = VERTICAL+10
Syringe = Syringe+5
Clear.to.warmer
Syringe.over.stirplate
Vertical = Vertical-2
Dispense = Syringe
Vertical = Vertical+3
Clear.to.warmer
Clear.to.water
Over.water
down.to.water
Syringe = Syringe+10
Over.water
clear.to.warmer
clear.to.water
Over.water
Syringe = Syringe+5
Clear.to.warmer
Syringe.over.stirplate
Vertical = Vertical-2
Syringe Syringe-20
Syringe Syringe-30
Vertical = Vertical+3
Clear.to.warmer
Clear.to.ice.bath
Remove.1ml.tip
Park.hand.4

EasyLab program: Get.hand.3

Coll.off
retry = 0
hand.error = 0
if hand.id = 3 then 30
if hand.id = 0 then 20
20 Wrist. = 0
Set.abs.at.hand.3
Into.hand.3
22 If reach.force > 0.7 then 25
Reach = Reach + 0.1
Retry = Retry + 1
if retry < 3 then 22
Goto 50
25 Above.hand.3
Clear.hand.3
30 Hand.id = 3
Goto 100
50 Before.hand.
Hand.id = 0
Hand.error
100 If coll.detection yes then coll.on
EASYLAB PROGRAM: WAIT.FOR.DATA.COLLECTION

DO 30 TIMES
SET TIMER 2 60 SECONDS
WAIT FOR TIMER 2
ENDDO

EASYLAB PROGRAM: CHECK.COOK.TEMPERATURE

10 RTDTESTFLAG 1
IF RTDTESTFLAG < 1000 THEN 10

EASYLAB PROGRAM: WAIT.FOR.COOK1

DO 30 TIMES
SET TIMER 2 60 SECONDS
WAIT FOR TIMER 2
ENDDO

EASYLAB PROGRAM: REMOVE.PROBE.FROM.SAMPLE

CLEAR.TO.WARMER
VERTICAL = 11
GRIP = 200
BEFORE.SPROBE
PROBE.IN.SAMPLE
GRIP = 7
PROBE.OVER.SAMPLE
CLEAR.TO.WARMER
CLEAR.TO.PROBE
RINSE.PROBE
OVER.PROBE
DOWN.TO.PROBE
GRIP = 200
BEFORE.PROBE
CLEAR.TO.PROBE

EASYLAB PROGRAM: REMOVE.FLEAKER.FROM.STIRPLATE

VERTICAL = 35
CLEAR.TO.WARMER
WRIST = 186
GRIP = 200
OVER.WARMER
GET.ABS.OVER.WARMER
VERTICAL == 20.7
VERTICAL VERTICAL - 3.2
GRIP = 30
GET.ABS.OVER.WARMER
VERTICAL = VERTICAL + 15
CLEAR.TO.WARMER

EASYLAB PROGRAM: RETURN.FLEAKER.TO.BATH
CLEAR.TO.ICE.BATH
TRANS.OFF
ICE.BATH.RACK.INDEX SAMPLE.NUM
ICE.BATH.RACK
VERTICAL = 15
VERTICAL = 10.7
GRIP = 200
ICE.BATH.RACK
CLEAR.TO.ICE.BATH
TRANS.ON
WRIST 0

EASYLAB PROGRAM: GET.1ML.TIP
CLEAR.TO.TIP
RETRY.TIP 1
20 SET.ABS.TIP.RACK
IF RETRY.TIP>5 THEN 60
R.OVER.TIP
TRANS.OFF
SYRINGE = 20
RACK.2 := INDEX J
RACK.2
VERTICAL = VERTICAL - 7
TEMP. VERT. FORCE = VERTICAL.FORCE
VERTICAL = VERTICAL - 1.7
VERTICAL = VERTICAL - 1
RETRY = 1
30 IF VERTICAL.FORC<TEMP. VERT. FORCE - 0.2 THEN 50
IF RETRY>= 3 THEN 40
VERTICAL VERTICAL - 1
RETRY = RETRY + 1
GOTO 30
40 VERTICAL = VERTICAL + 10
    J = J + 1
    RETRY.TIP = RETRY.TIP + 1
GOTO 20
50 TRANS.ON
VERTICAL VERTICAL+10
REACH = 0
IF RACK.2.INDEX > 50 THEN RACK.2.INDEX = 1
GOTO 70
60 TIP ERROR.MESSAGE
70

EASYLAB PROGRAM: RINSE.PROBE

OVER.RINSE.STATION
DO 5 TIMES
DOWN.TO.RINSE.
UP.TO.RINSE
ENDDO
SET TIMER 1 10 SECONDS
WAIT FOR TIMER 1
OVER.RINSE.STATION

EASYLAB PROGRAM: RINSE.PROBE.ONCE

OVER.RINSE.STATION
DOWN.TO.RINSE
UP.TO.RINSE
SET TIMER 1 10 SECONDS
WAIT FOR TIMER 1
OVER.RINSE.STATION
CLEAR.TO.PROBE

EASYLAB PROGRAM: CHECK.INCUBATION.TEMPERATURE

10 FLAGTEST 1
    IF RTDFLAG < 300 THEN GOTO 10

EASYLAB PROGRAM: GET.HAND.5

COLL.OFF
RETRY = 0
HAND.ERROR 0
IF HAND.ID = 5 THEN 30
IF HAND.ID = 0 THEN 20
PARK.HAND
20 WRIST = 0
SET.ABS.AT.HAND.5
BEFORE.HAND.5
BEFORE.HAND.5
INTO.HAND.5
22 IF REACH FORCE > .7 THEN 25
REACH = REACH + 1
RETRY = RETRY + 1
IF RETRY < 5 THEN 22
GOTO 50
23 ABOVE.HAND.5
CLEAR.HAND.5
70 HAND.TO 5
GOTO 100
50 BEFORE.HAND.5
HAND.ID 0
HAND.ERROR.999
100 IF COLL.DETECTION YES THEN COLL.ON

EASYLAB PROGRAM: REMOVE.FLEAKER
CLEAR.TO.WARMER
WRIST
GRIIP
APPORACH.WARMER
OVER.WARMER
DOWN.TO.WARMER
GRIIP
OVER.WARMER
CLEAR.TO.WARMER
CLEAR TO ICE.BATH
APPROACH.ICE.BATH
OVER.SAMPLE DOWN.TO.SAMPLE.
GRIIP
OVER.SAMPLE
CLEAR TO ICE.BATH

EASYLAB PROGRAM: STIR.CULTURE
STIRPLATE = 200
GET TIMER 1 60 SECONDS
WAIT FOR TIMER 1

STIRPLATE = 0
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