Evaluation of Formagraph for Comparing Rennet Solutions[1,2]

DONALD J. McMAHON and RODNEY J. BROWN
Department of Nutrition and Food Sciences
Utah State University
Logan 84322

ABSTRACT

A new instrument for measuring milk clotting, the Formagraph, was evaluated. Measurement is movement of small pendulums immersed in linearly oscillating samples of coagulating milk. Pendulum movements are recorded on photographic paper, producing a diagram of firmness versus time. The instrument can be used for measuring milk-clotting enzyme activity in comparison to a standard, and in constrast to the rolling bottle method, it does not require continual observation. Coagulation times measured by the Formagraph are longer than those measured by the rolling bottle method, but both methods give linear standard curves between .2 and 1.0 rennet units/ml enzyme concentrations.

INTRODUCTION

Numerous methods have been used to measure rennet activity (4). The most common method depends upon visual observation of the formation of a clot, or rather the sudden fracture of a film of milk on the wall of a bottle or test tube (2, 8). An ideal way to measure milk-clotting activity of an enzyme, however, has yet to be devised. Unlike usual procedures in enzyme chemistry, milk clotting activity may be measured only by the rapidity with which the enzyme clots milk under a set of specified conditions.

The Formagraph is an instrument designed to record coagulation properties of cheese milk. The technique by which the Formagraph determines coagulation is based upon movement of small, stainless steel, loop pendulums immersed in linearly oscillating samples of coagulating milk (Figure 1).

Minute forces are applied to the pendulums as a consequence of formation of a gel in the moving milk sample. A light flash at each end of the sample oscillation records the pendulum position on self-developing photographic paper. The result is a diagram of firmness versus time. The light flashes are timed at 4/min to coincide with the limits of the oscillation strokes.

The Formagraph and the rolling bottle method described by Sommer and Matsen (8) were compared for measuring milk-clotting enzyme activity. The viscosity of milk at which coagulation is detected by the two methods was compared with the method of Kopelman and Cogan (6).

MATERIALS AND METHODS

Reagents

Low-heat nonfat dry milk was reconstituted in .01 M $CaCl_2$ (1) and allowed to equilibrate at 5°C for 20 h (3). Prior to each analysis the milk substrate was warmed to 30°C and maintained at that temperature for 30 min.

A purified calf rennet solution of nominal clotting activity of 100 rennet units (3) per milliliter (RU/ml) was obtained from The New Zealand Cooperative Rennet Co., Ltd., Eltham, New Zealand. All dilutions were with distilled water and maintained at below 2°C throughout the experiment.

Formagraph Method

The Formagraph (5) consists of two modules. A service module heats the sample cuvettes and milk, controls the temperature of the instrument, and houses the on/off controls for up to five recorder modules. The recorder module consists of a ten-channel recording system. Each channel comprises a pendulum with counterbalanced damper and an adjacent

Received October 19, 1981.

[1] Utah Agricultural Experiment Station Journal Article No. 2736.

[2] Formagraph supplied by Foss America Inc., Fishkill, NY 12524.

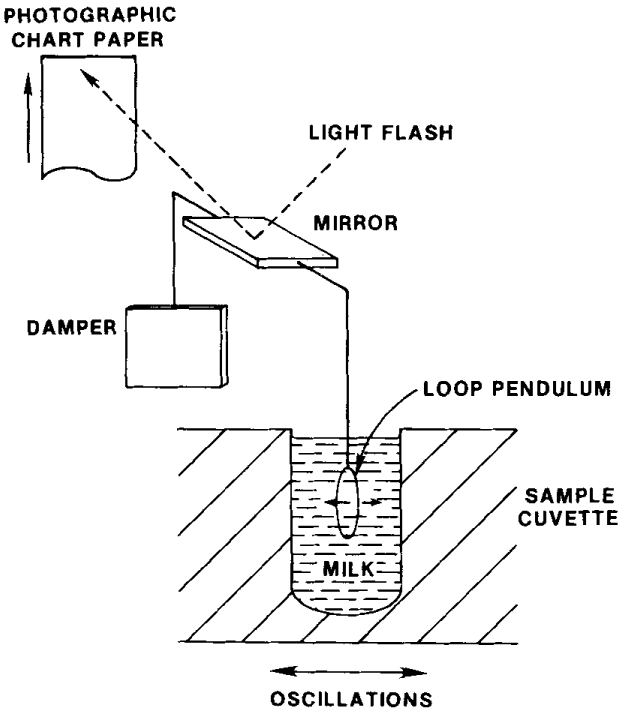

Figure 1. Schematic diagram of the Formagraph instrument for measuring and recording coagulation of milk.

optical system. Common to all channels are the sample oscillator system, light flashing unit, and strip chart recorder.

Sample cuvettes are placed on the service module heating plate, and 10 ml of substrate is deposited in each sample well. Enzyme solution (200 μl for each sample) is pipetted into a multiple spoon apparatus to allow simultaneous dispensing of enzyme into all samples. The recorder module is started the instant the enzyme is added to the milk. Samples are stirred with the spoons; then the cuvette is transferred to the recorder module.

Samples are brought into contact with the pendulum loops. While the milk remains uncoagulated, insufficient force is transmitted to the pendulum from the linearly oscillating milk to cause the pendulum to move. When coagulation occurs, the resultant increase in viscosity and formation of a curd causes synchronous motion of the pendulum, and light flashes reflected from the pendulum mirrors are recorded at different lateral positions on the chart.

In a typical diagram of firmness versus time (Figure 2), r represents time until formation of a gel. The value of r was determined by measuring distance from the origin to the point where the baseline began to increase in width. The time from the start of gel development until a width of 20 mm is reached on the chart is shown as k_{20}. This equates with a curd firmness adequate for cutting of cheese curd. It has been suggested that by keeping enzyme concentration constant, the measurement a_{30} can be used for comparing cheese milk samples (5). This is the width of the graph 30 min after enzyme is added.

Milk clotting is influenced by numerous factors including temperature, substrate composition, and enzyme concentration. The effect of each of these can be examined by holding all of the others constant.

Rolling Bottle Method

The apparatus described by Sommer and Matsen (8) was used. One-half milliliter of rennet solution was added to each 25-ml portion of Berridge substrate (1) in 125 ml wide mouth bottles. The analysis was at 30°C.

Viscosity Measurement

Viscosity was measured by the procedure of Kopelman and Cogan (6).

Statistical Design and Analysis

A randomized block design was used to eliminate effects of possible changes in standard enzyme activity during the experiment. Both instruments accommodated 10 samples simultaneously, which permitted duplicates of five enzyme concentrations within each block.

Measurements of coagulation time were

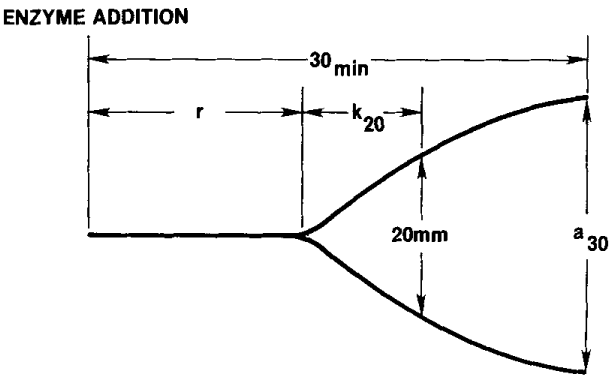

Figure 2. Diagram of coagulation and curd firmness as a function of time as recorded with the Formagraph.

TABLE 1. Analysis of variance of Formagraph method versus rolling bottle measurements of milk coagulation at various rennet concentrations (blocking over time).

Source	DF	MS	F	Significant α
Block	4	1.68	....	....
Rennet	4	2072.16	12228	.0001
Method	1	82.70	488	.0001
Rennet X method	4	5.22	31	.0001
Residual	67	.17		

simultaneous on the two instruments. If a mistake occurred in preparing or recording a sample, then all measurements at that rennet concentration in that block were discarded to maintain balance between the two methods. Analysis of variance and linear regressions were by SAS statistical package (7).

RESULTS AND DISCUSSION

Comparison of Methods

There was a difference ($P \leq .0001$) in coagulation time by the two methods (Table 1). Separate analyses of variance at each rennet concentration also showed significant differences in coagulation time for the two methods. The interaction between method and rennet concentration was also significant. At lower rennet concentrations, the rate of curd formation (once coagulation has started) was slower (Figure 3). As coagulation time was extended, the Formagraph endpoint fell further

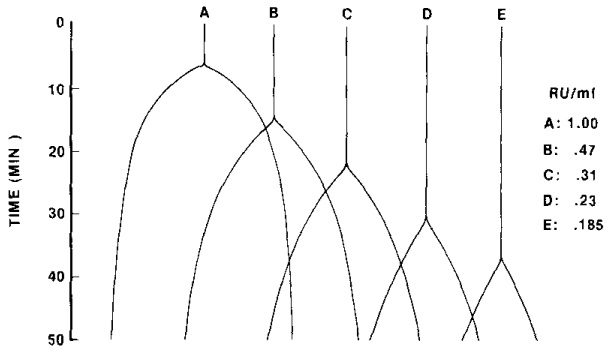

Figure 3. Coagulation and curd firmness as a function of time at various rennet concentrations.

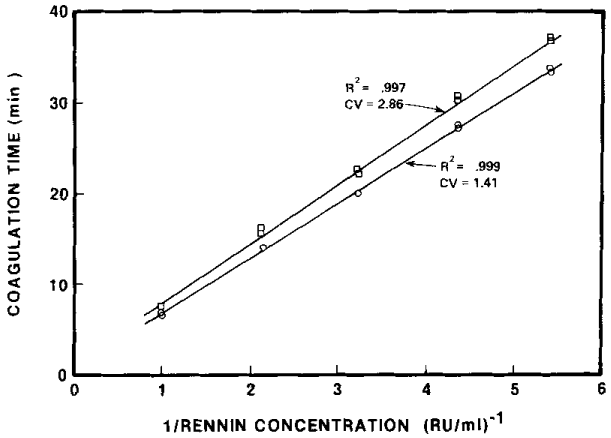

Figure 4. Linear regressions of coagulation time versus inverse of rennet concentration. Both methods were used simultaneously with duplicate samples at each concentration. □ Formagraph; ○ rolling bottles.

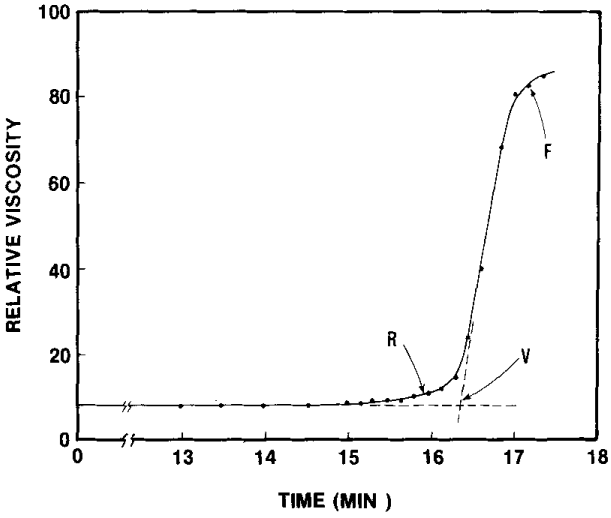

Figure 5. Viscosity versus time for milk-rennet system (2% of .40 RU/ml in Berridge substrate) showing measurement of coagulation by three methods: Formagraph (F); rolling bottles (R); and viscosity (V).

behind that of the rolling bottle method.

Linear regressions of the inverse of actual rennet concentrations versus measured clotting times for both methods (Figure 4) yielded $R^2 > .995$. Both R^2 were essentially the same, which indicates that the two methods are equally useful for measuring coagulation time as a function of enzyme activity in comparison to a standard enzyme solution. Coefficients of variance (CV) of mean coagulation times for each method are shown in Figure 4, indicating more variance in Formagraph measurements than in rolling bottle measurements.

Viscosities of milk at the point of coagulation as measured by the two methods were compared (Figure 5). The coagulation point for the Sommer and Matsen rolling bottle method, which is defined as the breaking of a milk film on the inner surface of the bottle (8), occurred prior to the coagulation point of the Formagraph. The latter requires minimal gel formation to induce pendulum movement before coagulation can be detected. The coagulation point in the Kopelman and Cogan viscosity procedure is defined as the extrapolated intersection of the two straight line portions of a plot of viscosity versus time (6). The Formagraph shows the progress of curd formation, whereas the rolling bottle method gives only an endpoint.

CONCLUSIONS

Coagulation time as measured by the Formagraph is linear with the inverse of rennet concentration within the five fold range of .2 to 1.0 RU/ml. Milk-clotting enzyme activity can be measured equally well by Formagraph or rolling bottle method for enzyme activity compared to a standard sample.

REFERENCES

1 Berridge, N. J. 1952. Some observations on the determination of the activity of rennet. Analyst 77:57.

2 Berridge, N. J. 1952. An improved method of observing the clotting of milk containing rennin. J. Dairy Res. 19:328.

3 Ernstrom, C. A. 1958. Heterogeneity of crystalline rennin. J. Dairy Sci. 41:1663.

4 Ernstrom, C. A. 1974. Milk-clotting enzymes and cheese chemistry. Part I. Milk clotting enzymes and their action. Pages 662–718 *in* Fundamentals of dairy chemistry. 2nd ed. B. H. Webb, A. H. Johnson, and J. A. Alford, ed. Avi, Westport, CT.

5 Foss & Co. 1980. Instruction manual for Formagraph 20–40–60–80–100. 66, Helleruprej-DK 2900 Hellerup-Denmark.

6 Kopelman, I. J., and U. Cogan. 1976. Determination of clotting power of milk clotting enzymes. J. Dairy Sci. 59:196.

7 SAS Institute. 1979. SAS Users guide. SAS Inst., Raleigh, NC.

8 Sommer, H. H., and H. Matsen. 1935. The relation of mastitus to rennet coagulability and curd strength of milk. J. Dairy Sci. 18:741.