A Study of Some of the Physical Changes Involved in the Rennet Coagulation of Milk and the Subsequent Firming of the Curd

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COMPARATIVELY little is as yet known about the actual physical and physico-chemical changes taking place in milk during and immediately following rennet coagulation. In this study some observations were made on the changes in viscosity and electrical conductivity during coagulation, as well as some ultramicroscopic observations. Special attention was, furthermore, given to the effect of various factors on the increase and final degree of curd firmness following rennet coagulation.

VISCOSITY

The findings of various previous investigators vary regarding the changes in the viscosity of milk during rennet coagulation. While some of them noted a definite gradual rise in viscosity between the time of adding the rennet and visible coagulation, others could not observe any definite increase in the viscosity of the milk before coagulation had occurred.

To further investigate this point, viscosity measurements were made by means of a Lawaczeck viscosimeter at one- to two-minute intervals during rennet coagulation. This instrument seemed to lend itself admirably for this work. In general, it was possible to confirm the work of Gutzeit (1), who, by means of a Reischauer viscosimeter, found that the viscosity of the milk began to rise sooner and continued more gradually at the lower temperatures and with the greater rennet concentrations than it did when higher temperatures and less rennet were employed. Under the latter conditions the rise was almost imperceptible at first but

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very sudden immediately before visible coagulation occurred. The results of our study seemed to indicate that the course of the viscosity curve is dependent upon the same factors which influence the firming of the curd after visible coagulation, so that it may be possible to anticipate the curd firming by an observation of the viscosity curve during coagulation.

The effect of aging the milk at room temperature on the viscosity increase was interesting. Skim milk was used in which the pH was kept nearly constant by the addition of mono-iodo-sodium acetate. A very stable, powdered rennet, carefully weighed each time on an analytical balance, was used and experiments were run in duplicate. Under these conditions aging definitely increased the coagulation time and delayed the rise in viscosity prior to curdling. Visible coagulation, however, always occurred at approximately the same viscosity, namely, 2.4 to 2.5 centipoises, so that the viscosity curves definitely flattened with each increase in the aging period of the milk. At the moment of visible coagulation there was always a slight reduction in viscosity which lasted only for an instant but was, nevertheless, very definite. This phenomenon might possibly be explained by the observation of Bleyer and Seidl (2) that the viscosity of calcium paracaseinate is lower than that of calcium caseinate.

The time necessary for the milk to reach a certain degree of viscosity was nearly proportional to the time required for coagulation. Thus, milk aged for 31 hours at room temperature required 39.5 minutes for coagulation compared with 20.5 minutes for the unaged milk, or about twice as long. The time required for the aged milk to reach a viscosity of 1.62 centipoises during coagulation was 24.5 minutes compared with 12.8 minutes for the unaged milk or, again, nearly twice as long. A viscosity of 2.26 centipoises was reached by the aged milk in 36 minutes and by the unaged milk in 18 minutes, again twice the time. The milk in these experiments was coagulated at 20° C.

ELECTRICAL CONDUCTIVITY

Few investigations are on record regarding the effect of rennet coagulation on the electrical conductivity of milk. Although no appreciable changes were recorded by any authors, fluctuations were noticed by Reichel and Spiro (3) during coagulation, and, as previously mentioned, Bleyer and Seidl (2) state that the electrical conductivity of calcium paracaseinate is greater than that of calcium caseinate.

The Kohlrausch apparatus with alternating current and telephone was used in this study together with a Wheatstone bridge. The resistance capacity of the Vessel (C) was ascertained by means of a saturated gypsum solution and found to be 0.322. This factor (C) divided by the resistance (R) found, gives the specific conductivity (K) of the milk ($K = C/R$). Results showed that the electrical conductivity of the milk was slightly raised by the addition of the rennet, due no doubt to the salt content of the latter. No change, however, was noted after the addition of the rennet except when the acidity was increasing in the milk. The specific conductivity found in various kinds of sweet raw and heated samples of milk ran between 57×10^{-4} mho and 65×10^{-4} mho, but in a sample of souring raw milk it increased from 71×10^{-4} mho to 92×10^{-4} mho in 105 minutes.

ULTRAMICROSCOPIC OBSERVATIONS

A few ultramicroscopic observations of the rennet coagulation of diluted skim milk were made by means of a Zeiss cardioid ultramicroscope. The observations revealed comparatively little. Coagulation seemed to consist of a heaping up of the casein particles accompanied by a gradual slowing up of an initially very active Brownian movement of these particles.

FIRMING OF THE CURD

In order to be able to follow closely the development of firmness in the curd, an instrument was devised in the nature of a lever balance, using no springs, which, when lowered down into the curd, measures the resistance offered by the curd in grams. The instrument was lowered into the curd contained in vessels of uniform size to a constant depth and at a uniform speed by means of an electric motor; thus the human factor was completely removed and measurements could be made at one- to two-minute intervals. The temperature of the coagulating milk and of the firming curd was kept constant in a large water bath in which temperature was controlled to $\pm$ 0.2° C. Hydrogen ion concentration in the milk was kept nearly constant for the duration of each experiment, and electrometric determinations were made at the beginning and end of each run. The procedure gave check results and proved quite accurate until sooner or later, according to the conditions of temperature, acidity, amount of rennet, etc., accurate reading became vitiated by peptic decomposition induced by the pepsin in the rennet.

To maintain a constant pH in the milk throughout each one of the experiments, which extended over several hours and which frequently were conducted at temperatures favorable for acid formation by microorganisms, a preservative was sought which would prevent bacterial activity and which at the same time would not change the pH of the milk nor affect the action of the rennet enzyme on the milk as regards coagulation time and firmness of curd. Toluol, chloroform, perhydrol, a solution of iodoform in acetone, as well as several other preservatives, proved unsuitable for these experiments, as the smallest amounts needed to prevent acid development usually retarded rennet coagulation or produced other undesirable effects. The most suitable preservative found was a .02 to .06 percent concentration of mono-iodo-sodium acetate. Usually

15 cc. of a 6 percent solution of this preservative was used in 2,000 cc. of milk.

AMOUNT OF RENNET AND CURD FIRMNESS

According to the law of Storch and Segelcke, coagulation time is reversely proportional to the quantity of rennet added; or, in other words, the product of coagulation time and rennet quantity is a constant. Within the range of the amounts of rennet commonly used in cheese manufacture, this law was found to be fairly accurate. However, over a considerable range of variation in the quantity of rennet used, the law could not be confirmed. It was found that the product of the quantity of rennet and coagulation time rises with rising rennet concentrations and the curd firmness produced per unit of rennet in a definite time after coagulation drops with increasing rennet concentrations, and the more so, the later the firmness is measured. Thus, the curd firmness produced 30 minutes after coagulation per unit of the particular rennet powder used decreased in the ratio of 111:100:62:17:4 with the following increase in rennet powder concentrations: 1:100,000; 1:50,000; 1:25,000; 1:5,000 and 1:1,000 (i. e., one part rennet powder to 1,000 parts of milk). Thus, it is seen that the efficiency of the rennet (considering curd firmness produced) when added in the high concentration of 1:1,000 is only 4 percent of that of the same rennet when added in a concentration of 1:50,000; and, when the rennet was used in the concentration of 1:25,000, it was only 62 percent as efficient as when used 1:50,000. When curd firmness as measured two hours after coagulation instead of 30 minutes after coagulation was used as a criterion of rennet efficiency, the drop in efficiency with increasing concentration was still more pronounced, being in the ratio of 137:100:44:11:2 for the above mentioned rennet concentrations. Rennet is, therefore, most efficient when added in low concentrations, except at 50° C., when the weakening effect of the heat exerts itself most strongly on the low concentrations of the rennet enzyme.

TEMPERATURE AND CURD FIRMNESS

As the temperature of coagulation was lowered from 45° C. to 20° C., a successive lengthening of coagulation time and reduction of curd firmness occurred. This weakening effect on the enzyme action grew with each successive lowering of the temperature. The difference in coagulating speed and curd firmness at 45° C., 40° C. and 35° C. was small, while at 25° C., 20° C. and 15° C. the effect of each lowering in temperature was very marked and curd firmness was affected more severely than coagulation time. At 50° C., compared with 45° C., coagulation time was lengthened due to a partial destruction of the enzyme at the higher temperature. Since the pepsin in the rennet seems to be less severely affected by the heat than rennin, peptization of the curd markedly reduces curd firmness at the higher temperatures. When curd firmness was measured from 25 to 40 minutes after coagulation, it actually decreased with each rise in temperature from 35° to 50° C. because of the continued influence of the pepsin action.

Maximum curd firmness was obtained at the lower coagulation temperatures; although, of course, the firming was very slow. Thus, the highest curd firmness (75 g.) was attained in a sample thermostatically held for 10 days at 10° C. Freezing the milk solid and holding it in a frozen state for three hours had no appreciable effect on the rennet coagulability of skim milk. In some experiments even a slight reduction of coagulation time was observed.

THE EFFECT OF AGING

Aging skim milk for 7, 26 and 77 hours at room temperature markedly retarded coagulation and the firming of the curd with each increase in holding time, although the pH value of the preserved milk dropped from 6.64 to 6.60. When the milk had been held 104 hours and the pH value had dropped to 6.57 the curd firmness curve, due to the lowered pH, ran slightly above that of the milk held 77 hours, but still considerably below the one of the milk held 26 hours. Aging affected in the same way the viscosity curves of the milk during coagulation.

THE EFFECT OF PASTEURIZATION OF THE MILK

Flash pasteurization of the milk at 85° C. retarded rennet coagulation and curd firming markedly; vat pasteurization at 62° to 63° C. for 30 minutes retarded it only slightly.

OTHER EFFECTS

Homogenization of the milk did not seem to reduce the rennet coagulability of milk, and homogenization of cold milk seemed even to improve it.

Shaking of milk and rennet for 20 seconds retarded coagulation, probably due to absorption of the enzyme in the foam. Churning skim milk for an hour both at 25° C. and at 40° C. retarded coagulation slightly and the firming of the curd markedly. Liquefied foam showed an improved coagulability in comparison with the milk from which it was obtained. The presence of foam in the coagulating milk of course reduces curd firmness.

Addition of gelatine to skim milk accelerated coagulation and curd firming, whereas *additions of albumin* had little effect.

Dilution of 10 percent with distilled water retarded coagulation and curd firming, while a 5 percent dilution was of only slight consequence.

Fat content within the range of normal variations in milk had no effect on coagulation and curd firming. *Increasing hydrogen ion concentrations* accelerated coagulation and curd firming; however, the kinds of acids, alkalies or salts used in changing the pH value was of great importance. Thus, coagulability and curd firmness were higher in a milk to which calcium salts had been added than when HCl or $LaCl_3$ had been added in amounts to produce the same pH value. Calcium salts seemed to be of specific value in increasing the coagulability of milk and the use of a salt with a cation of greater valence, such as $LaCl_3$, seems to exert no added influence. NaOH reduced coagulability and curd firmness more than $Ca(OH)_2$ at the same pH.

TIME AFTER COAGULATION

At the lower temperatures curd firmness increased for a considerable time proportional to the time after coagulation, while at the higher temperatures the firming of the curd proceeded rapidly at first, but soon slowed up, especially with the higher concentrations of rennet, most likely because of the interference of pepsin action.

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