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NON-DESTRUCTIVE VISCOMETRIC STUDIES OF ENZYMIC MILK COAGULATION III. THE EFFECT OF pH, TEMPERATURE AND CA-IONS CONCENTRATION ON THE SECONDARY PHASE OF MILK COAGULATION

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Key words: milk coagulation, immobilized rennin, Ca-ions.

It was observed that within the pH range between 5.57 and 6.37 the time of development of the secondary phase of the rennet milk coagulation is independent from temperatures between 25° and 35°C. Increased concentration of Ca-ions between 0.66 and 1.1 mg $CaCl_2$/ml milk accelerates the formation of the secondary phase.

INTRODUCTION

Application of immobilized rennin makes it possible to study the secondary (coagulation) in phase of milk because when the enzymatic phase is over the immobilized enzyme can be separated from milk. The presence of the enzyme in the reaction milieu disturbs the secondary phase of enzymatic coagulation of milk which is induced by coagulation of paracasein in it [4]. The mechanism of the enzymatic coagulation of milk has not so far been very clear [4, 7, 8].

The paper is an attempt at characterizing the effect of Ca-ions and H-ions concentration and temperature on kinetics of the secondary phase of enzymatic coagulation of milk with the use of immobilized rennin.

MATERIALS AND METHODS

In the studies a solution obtained from fat free dried milk Instant (12 g milk in 100 ml 0.01 MCaCl) was used. The milk's pH was set with 2M H_3PO_4 or 1N NaOH. The level of $CaCl_2$ in milk was changed within

0.66-1 lmg $CaCl_2$/ml milk. The immobilized rennin preparation was obtained with the radiation method [6], in poliacrylamid gel used as a carrier. The preparation was washed several times with distilled water to remove the soluble enzyme. The enzymatic reaction was performed in a reactor with a mixer at 15°C thermostatically controlled.

To a sample of milk (10 ml) was added 1 mg enzyme per 1 ml milk. The reaction was continued for 20 min. with intensive stirring, then, after separation from the immobilized enzyme, 5 ml milk was immediately transferred to the thermostat vessel at 25°, 30° or 35° to measure viscosity with a viscosimeter provided with an ultrasonic niddle [1, 2].

RESULTS AND DISCUSSION

The effect of pH on the secondary enzymatic phase of milk coagulation. The data on the voltage signal in the ultrasonic viscosimeter during the secondary phase of enzymatic milk coagulation at pH 5.65 to 6.37 at 30°C are given in Fig. 1.

Fig. 2 presents the time dependence concerning the start of the secondary enzymatic phase of milk coagulation and the level of PH (The time of formation of the secondary phase at pH = 6.14 was taken as 100%). With measurements at 25°, 30° and 35°C at a settled dose of the immobilized enzyme it was observed that the change of the relative time of formation of coagulated matter at growing levels of pH does not depend on temperatures between 25° and 35°C. This points to a considerable

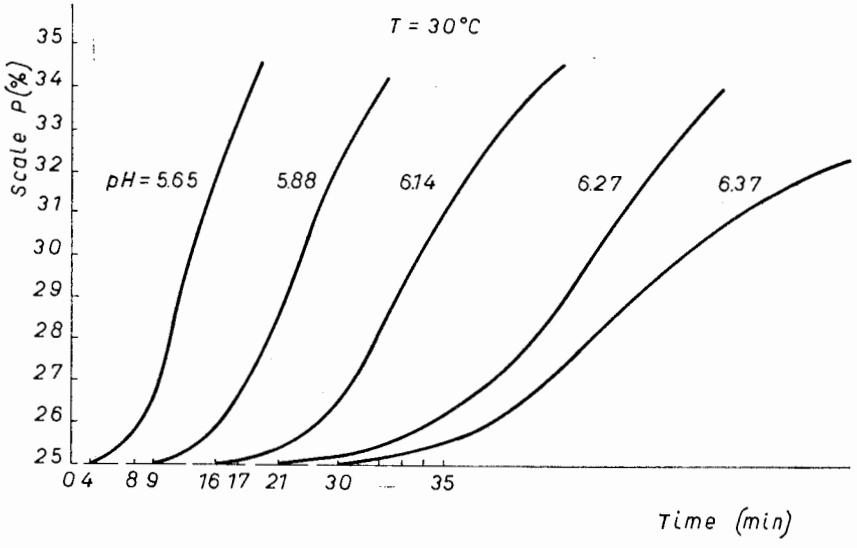

Fig. 1 Voltage signal in the ultrasonic viscosimetr during the secondary phase of enzymatic milk coagulation at 30°C for pH levels of 5.65 to 6.37

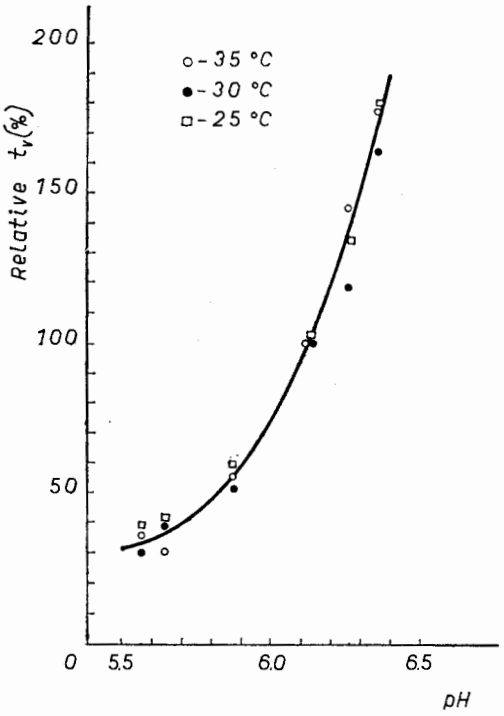

Fig. 2 Dependence of the relative time of formation of the second phase of enzymatic milk coagulation on pH levels of 5.57 to 6.37 at 25°, 30° and 35°C

participation of hydrogen ions in the process of coagulation of paracasein in milk within this temperature range.

Fig. 3 presents the changes in viscosity during the secondary phase of (—) enzymatic milk coagualation. It was calculated with the parameters presented in Fig. 3 as to the value of velocity constant K of the paracasein coagulation process. It is equal to the slope of the straight-line section of the curve.

Fig. 4 is a curve of dependence of the log constant K on pH levels. The dependence makes it possible to calculate the order of the paracasein coagulation process in milk in relation to the concentration of H-ions, according to the following equations (1) — (4):

$$v_0 = k \cdot C_0^{\varkappa} \tag{1}$$

v_0 — initial velocity of the coagulation reaction

$\varkappa$ — paracaseins in milk in the secondary phase of enzymatic coagulation at determined pH levels

C_0 — concentration of $\varkappa$-casein decomposed in the enzymatic phase. The concentration is constant when temperature, pH, and time of enzymatic reaction are constant.

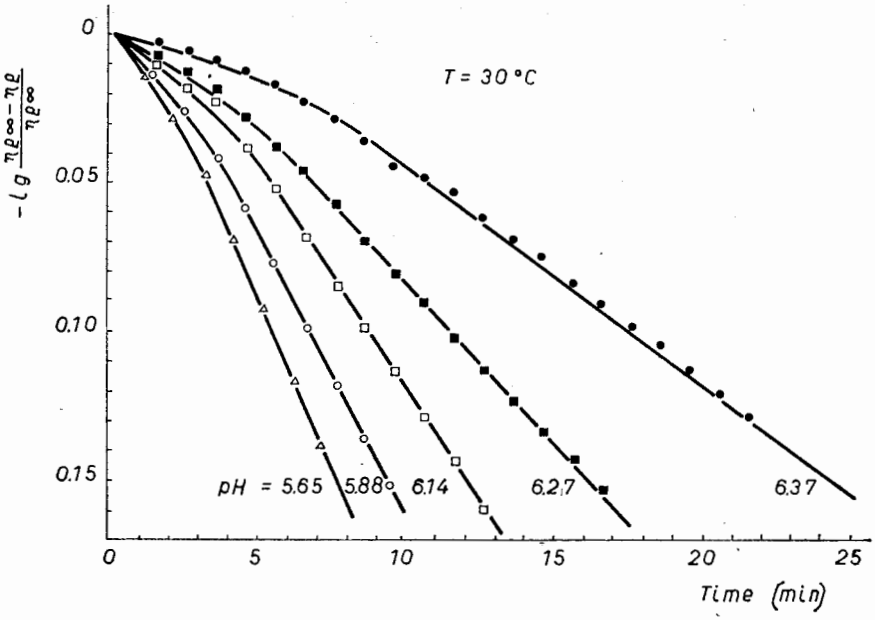

Fig. 3 The changes in viscosity during the secondary phase of enzymatic milk coagulation at 30°C for pH levels of 5.65-6.37

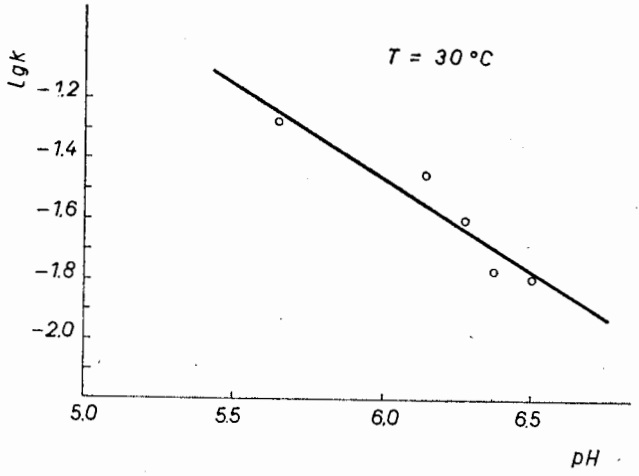

Fig. 4 Dependence of log constant of the maximum rate of the secondary phase of enzymatic milk coagulation at 30°C on pH levels of 5.65 to 6.37

α — order of reaction (equal to the data in the former study [2]

Having taken into consideration the influence of (H^+) on v_o, equation (1) becomes:

$$V_0 = k'(H^+)^n C_o \qquad (2)$$

And then:

$$K = k'(H^+)^n \tag{3}$$

according to (1), (2) and (3) equations, the dependence in Fig. 4 may be described as follows:

$$\log k = \log k' - npH \tag{4}$$

The slope of the straight line, calculated from the curve, is 0.59. The n value defines the order of the milk paracasein coagulation process in relation to concentration of H-ions.

Fig. 5 gives the dependence of the log maximum rate of increasing milk viscosity $\left[\frac{\Delta\eta\rho}{\Delta t}\max = V_0\right]$ on pH levels. It was observed that the value of maximum rate of viscosity increase is reduced with growing pH for three temperatures: 25°, 30° and 35°C. The inclinations of the straight lines in Fig. 5 was used to determine the order of the milk paracesin coagulation process „n" in relation to concentration of H-ions.
According to equations (1) — (4),

$$\log V_0 = \log k'C_0 + n \log [H^+] \tag{5}$$

$$\log \frac{\Delta\eta\rho}{\Delta t} = A - npH,\ A = \log k'Co_0 \tag{6}$$

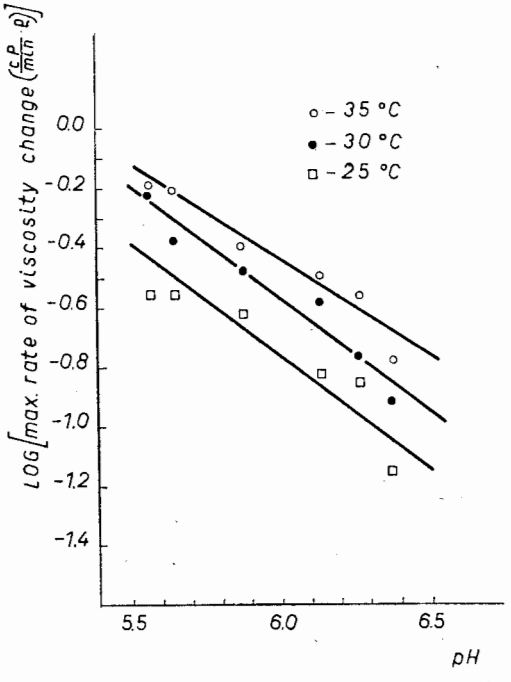

Fig. 5 Dependence of log maximum rate of increasing viscosity for pH Levels of 5.57 to 6.37 for 25°, 30° and 35°C

The „n" value for 25°, 30° and 35°C is 0.68.
In both experiments, therefore, approximately the same values — ca 0.5 — of the order of reaction were arrived at.

During the enzymatic phase ϰ-casein changes into ϰ-paracasein [4]. In the secondary phase the formed ϰ-paracasein binds Ca and soluble phosphates (radical of phosphoric acid) building an insoluble complex. Mechanism of the secondary phase of enzymatic coagulation of milk is probably based on reaction of neutralization of the charge of renneted casein micelles. Using the calculated order of reaction in relation to H-ions concentration and to concentration of reneted casein micelles, stoichiometry of this reaction is probably the following:

$$P^- + 1/2HA \rightarrow 1/2HP_2^- + 1/2\,A^-$$

P^- — molecule of ϰ-casein in milk after enzymatic hydrolysis, HA — acid molecule.

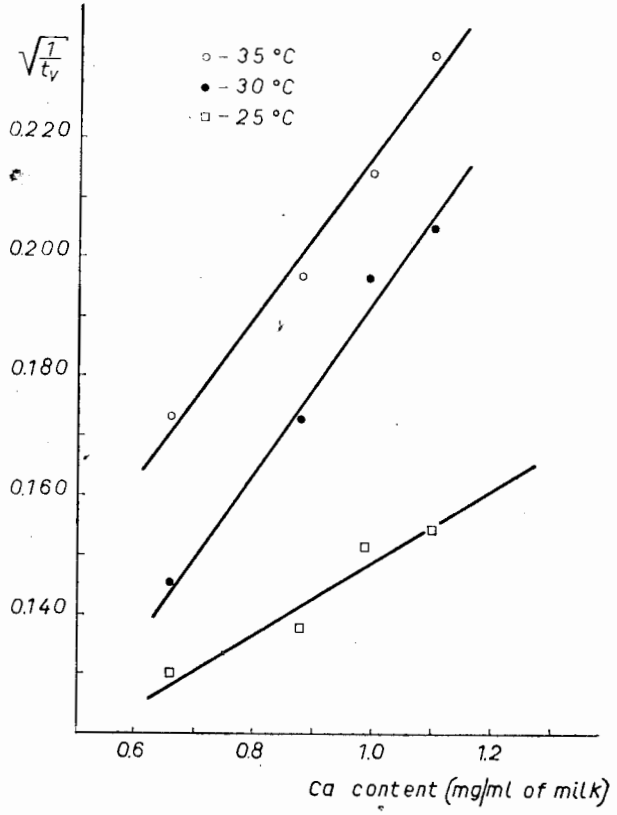

Fig. 6 Dependence of the inverse value of the time of the secondary phase of enzymatic milk coagulation on concentration of the Ca^{2+} ions in milk between 0.66 and 1.1 mg/ml for 25°, 30° and 35°C

THE EFFECT OF CONCENTRATION OF CA-IONS ON THE SECONDARY PHASE OF ENZYMATIC COAGULATION OF MILK

One of the processes in the secondary enzymatic phase is cross linkage of metastable casein micelles formed in the enzymatic phase by Ca-ions in milk [5].

The curves in Fig. 6. show the dependence of inverse data of the time of formation of the secondary phase on concentration of Ca-ions in milk at 25°, 30° and 35°C.

The formula $\frac{1}{t_v} = f(Ca^{2+})$ has a parabolic shape $\sqrt{\frac{1}{t_v}} = f(Ca^{2+})$ was applied to obtain linear functions [9].

Results of mathematical analysis of the investigated dependencies are given in Table. Values of the „r" coefficient in the table point to existence of a close correlation between the points of simple regressions and the measured values. The „a" coefficient of the simple regression makes the root of the inverse data on the secondary phase time depend on concentration of Ca-ions in milk. The value of this coefficient is approximately the same for 30° and 35°C.

This is an evidence of a similar influence of the Ca-ions concentration in milk on the time after which the secondary phase occurs at these temperatures. On the other hand, the „a" coefficient for 25°C is twice smaller than the coefficients „a" at 30° and 35°.

Table. The lines of regression of the $y = ax+b$ formula describing the dependence of the time of formation of the secondary phase of enzymatic milk coagulation on concentration of Ca-ions

Secondary phase temperature	$\sqrt{\frac{i}{t_v}}$ ($min^{-1/2}$) as a function of concentration of Ca^{2+} (mg/ml)	
	formula of the line	r
25°C	$y = 0.061x+0.088$	0.987
30°C	$y = 0.145x+0.049$	0.980
35°C	$y = 0.136x+0.083$	0.979

CONCLUSIONS

1) At pH levels from 5.57 to 6.37, the time of start of the secondary phase of enzymatic milk coagulation does not depend on temperatures between 25° and 35°C.

2) Increasing pH within the above range leads to the lowering of the maximum rate of changes in milk viscosity.

3) The constant of the rate of process in the secondary enzymatic phase of milk coagulation in relation to concentration of H-ions is expressed in the following equation:

$$k = k' (H+)^{0.5}$$

4) Increased concentration of Ca-ions in milk between 0.66 — and 1.1 mg $CaCl_2$/ml milk shortens the time of formation of the secondary coagulation phase.

The effect of concentration of Ca-ions in the above range on the secondary phase of enzymatic milk coagulation is particularly perceivable at 30° and 35°C

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NIE NISZCZĄCE BADANIA WISKOZYMETRYCZNE ENZYMATYCZNEGO PROCESU KRZEPNIĘCIA MLEKA. III. WPŁYW STĘŻENIA JONÓW WAPNIOWYCH, pH I TEMPERATURY NA WTÓRNĄ FAZĘ ENZYMATYCZNEGO KRZEPNIĘCIA MLEKA

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Streszczenie

Kontynuując wcześniej prowadzone prace na temat enzymatycznego procesu krzepnięcia mleka [1, 2] w tej części przedstawiono badania wpływu pH oraz stężenia jonów wapniowych we współdziałaniu z temperaturą na wtórną fazę enzymatycz-

nego krzepnięcia mleka. Do badań zastosowano unieruchomioną renninę. Stwierdzono, że dla wartości pH od 5,57 do 6,37 czas wystąpienia fazy wtórnej jest niezależny od temperatury w zakresie 25-35°C.

W miarę zwiększenia wartości pH następował spadek szybkości zmiany lepkości mleka w okresie wtórnej fazy. Wyznaczono zależność stałej szybkości zmian lepkości od stężenia jonów wodorowych: $k = k'(H+)^{0,5}$. Określono zależność odwrotności czasu wystąpienia fazy wtórnej od stężenia jonów wapniowych w granicach 0,66-1,1 mg $CaCl_2$/ml mleka dla temperatur w zakresie 25-35°C. Stwierdzono przyspieszenie wystąpienia fazy wtórnej w miarę wzrostu stężenia jonów wapniowych w mleku.