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NON – DESTRUCTIVE STUDY OF ACID MILK COAGULATION

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Abstract

The acid coagulation is the main processing step in the yoghurt production and has a great impact on yogurt texture, microstructure and rheology, contributing to the overall sensory and visual product perception by the consumer. The objective of this paper was to study the different stages of acid gel formation by turbidimetric and conductometric methods. The non-destructive methods were used to allow the differentiation of three regions: latent phase (pH = 6.3 ÷ 6.0), exponential phase (pH = 5.9 ÷ 5.4) and phase of protein structure formation of demineralized casein micelles (pH = 5.3 ÷ 4.6). The mathematical model obtained by the three-parameter sigmoid function can be used in future studies to predict the change in conductivity during acid coagulation.

Key words: *turbidimetric method, conductometric method, acid coagulation*

INTRODUCTION

The acid coagulation is the main processing step in the yoghurt production. This process of acid gel formation has a considerable impact on yogurt texture, microstructure and rheology, contributing to the overall sensory and visual product perception by the consumer.

The gradual accumulation of lactic acid and respective decrease in pH, significant physical and chemical changes in micellar structure of casein are observed. The acidification of milk disturbs the internal structure of casein micelles, which results from the dissolution of colloidal calcium phosphate (Dalgleish & Law, 1982).

As reported by Lucey (2004), the physicochemical mechanism of acid gel formation can be divided at three pH range:1) 6.7 ÷ 6.0; 2) 5.8 ÷ 5,0 and 3) ≤ 4.8.

By lowering the pH from 6.7 to 6.0 the negative charge of casein micelles decreases, which leads to a weakening of electrostatic repulsion (Lucey, 2007; Phadungath, 2005). At this pH range no change in the size of the casein micelles is observed since only a small portion of the colloidal calcium phosphate is dissolved at pH> 6.0.

At pH values between 5.8 and 5.0 a significant amount of colloidal calcium phosphate dissolves, which causes the weakening of the intracellular interactions between the casein fractions and increasing the size and degree of hydration of the micelles.

Lowering the pH to 4.6 (the isoelectric point of casein) leads to a weakening of electrostatic repulsion between casein molecules and an increase in attraction forces between casein particles (Horne, 1998). As a result of the physical and chemical changes in micellar structure, it is formed a three-dimensional protein network of clusters and chains of demineralized casein micelles (Lucey, 2004).

The objective of this paper was to study the different stages of acid gel formation by turbidimetric and conductometric methods. The change in the optical properties of milk during acid coagulation as well as the change in electrical conductivity allows the use of these physical methods for the study the overall process of formation of the acid gel.

MATERIAL AND METHODS

Starter culture

Commercial freeze-dried yogurt cultures (Laktina, Bulgaria) which are a mixture of Streptococcus salivarius subsp. thermophilus and Lactobacillus delbrueckii subsp. bulgaricus were used (LAT BY 8).

The low fat ultra – high temperature (UHT) processed cow's milk was incubated at 45 °C which is the optimal growth temperature of the strains. The process was considered finished when pH value of 4.6 was reached.

Measurement of pH

The pH was measured using a pH meter equipped with a combined electrode (Schott instruments, GmbH, Deutschland, Germany). The pH meter was calibrated with standard buffer solutions of pH = 4.0 and 7.0 before use. The pH was monitored until the pH value of 4.6 was reached (isoelectic point of casein).

Turbidity monitoring

Turbidity experiments were carried out in a 2 L vessel (5) connected to a circulating water bath (6) at 45 °C. The changes in turbidity (Δτ) of milk were monitored by a portable turbidity meter (3) (McVanAnalite NEP 160 Series, Mulgrave, Australia).This device uses near-infrared (NIR) light (860 nm), allowing each particle in the fluid to reflect the falling beam at 180˚. The reflected beam at 180˚ is captured by a second fibre optic network and transmitted to turbidity sensor (7) that transforms the signal into Nephelometric Turbidity Units (NTU).

The recording of data was performed using a Data logger (Almemo 8990-8V5, Ahlborn, Holzkirchen, Germany), connected to a personal computer.

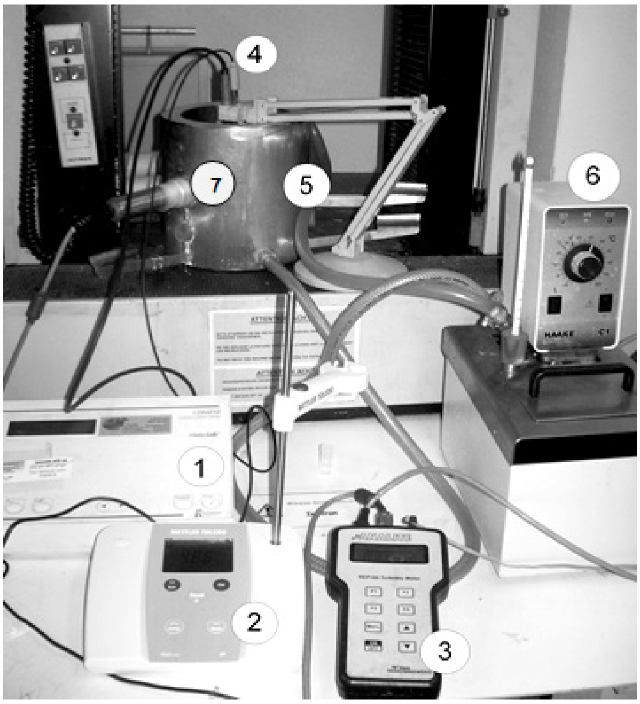

Figure 1. Experimental setup of acid milk coagulation by using aturbidity meter and a conductivity meter (1 – conductivity meter, 2 - pH meter, 3 – turbidity meter, 4 - pH electrode, 5 - thermostatic vessel, 6 - thermostat, 7 – turbidity sensor).

The change in the turbidity ($\Delta\tau$) was calculated as a difference between turbidity (τ) at time (t) and initial milk turbidity (τ_0) (OuldEleya et al.,1995).

$$\Delta\tau = \tau - \tau_0$$

where:

$\Delta\tau$ is the change of turbidity, NTU;

τ_0 - initial turbidity of the milk before inoculation, NTU;

τ - turbidity of milk at time t, NTU.

The first derivative ($d\tau/dT$) of the turbidimetric profile was calculated as a function of time using 40 data points. The second derivative ($d2\tau/dT$) was calculated using a similar procedure. All measurements were carried out at least in duplicate.

Conductometric monitoring

The electrical conductivity (G, $mS.cm^{-1}$) was measured using a conductivity meter (model CDM 210, Radiometer Analytical SAS, France). The conductivity meter was calibrated in the range of 0÷10 $mS.cm^{-1}$ by using a KCl solution.

Numerical differentiation of the pH and electrical conductivity data was performed to determine the rate of change of these parameters over time.

Statistical analysis

The statistical analysis, mathematical modelling and graphs were performed using Sigma Plot v.11.0. The resulting data was processed by dispersion analysis at significance level $p = 0.05$.

RESULTS AND DISCUSSION

Monitoring of acid coagulation by turbidimetric method

The change in milk turbidity during coagulation due to the increase in the size of casein micelles justifies the use of this optical method in the study of the process and its individual phases.

The numerical differentiation of the turbidity data ($\Delta\tau$) obtained during the acid coagulation with LAT BY 1-8 is shown in Figure 2-B.

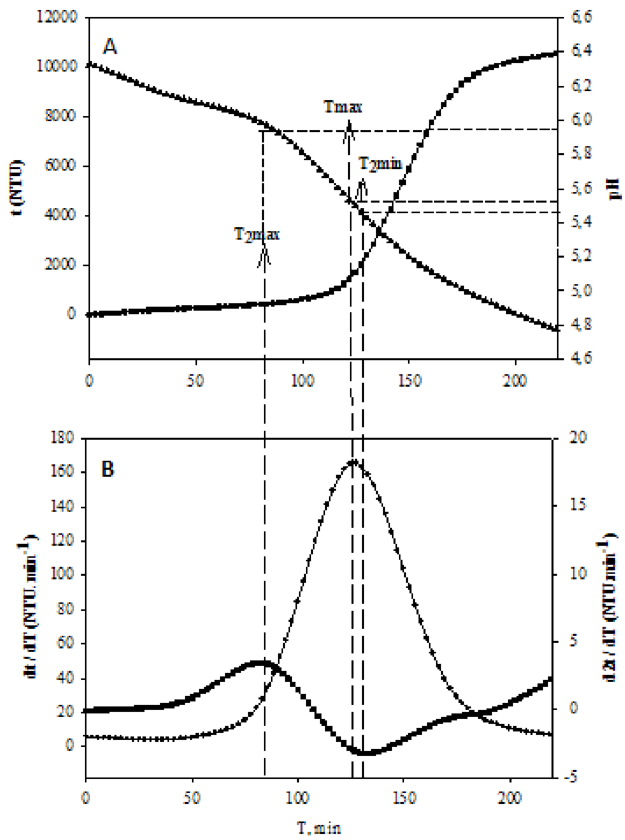

Figure 2. Change of $\Delta\tau$ (■) and pH (▲) during the acid coagulation of milk inoculated with a starter culture LAT BY 1-8 at 45 °C (A). First (dt / dT) (■) and second (d2t / dT) (●) derivative of $\Delta\tau$as a function of time (B).

Based on the identified inflection points (Tmax, T2max and Tmin) of the curve of turbidity (Δτ) can be differentiate three phases characterized by different dynamics in the velocity and acceleration of the ongoing process (Figure 2-A).

The first phase can be conventionally defined as a latent phase during which a slight change in Δτ was established. This change was in the order of 420 NTU (at baseline turbidity 1 NTU) for a time of 40 min. This phase was set at a pH values between 6.3 and 6.0.

In agreement with Lee and Lucey (2004), lowering the pH from 6.6 to 6.0 results in a reduction in electrostatic repulsion between casein micelles as a result of a decrease in their surface negative charge.

The second phase (pH = 5.5) of acid coagulation can be determine as an exponential phase and corresponds to a rapid increase of the values of the nephelometric units (NTU). This change can be explained by the increase in the size of the case in micellesin the indicated pH range. The significant increase in turbidity at the pH range of 5.4 ÷ 5.5 was also found by McMahon (2009) in a spectrophotometric study of acid coagulation at 40 °C.

After reaching the Tmax it was set up a delay in the rate of Δτ (T2min) at pH = 5.4. At this pH range the third phase is observed during which a protein structure of demineralised casein micelles is formed. At pH 5.2 ÷ 5.0 a significant amount of colloidal calcium phosphate dissolves (Dalgleish, 1989), which leads to weakening of the micellar interactions between casein fractions. In the pH range of 5.3 ÷ 4.6 the spatial structure characteristic of acid gels is finally formed, and thus the beginning of the last phase of the gel formation.

Similar changes in the turbidimetry profile of acid coagulation were also found by Bringe and Kinsella (1990) at 25 °C, Banon and Hardy (1991; 1992) at 30 and 42 °C and McMahon (2009) at 40 °C.

Monitoring of acid coagulation by conductivimetric method

The resulting curves for the change in active acidity and conductivity as well as their first derivatives (dpH / dt = f (t) and dG / dt = f (t)) are shown in Figure 3 and 4.

The pH curve (Figure 3) shows a decrease in its forming values, whereas that of the electrical conductivity (Figure 4) has an increasing profile. This is related to the amount of lactic acid produced, which leads to a decrease in pH and increase in the electrical conductivity (G) of milk due to the accumulation of H^+ and lactate ions during the lactic acid fermentation.

At pH 6.1 ÷ 6.2 for a period of 40 ÷ 45 min there was observed a slightly delay in the acidification rate ((dpH/dt)1) and up to 80 min had no effect on it. After this time interval, the second inflection point ((pH/dt)2) was established, indicating the attainment of the highest rate of reduction of the active acidity. According to the data obtained, the velocity was significant in the range of 110 ÷ 115 min, and the H^+ concentration achieved was 5.6 (Figure 3).

The highest rate of acid formation was reached after a period of 70 min into account the maximum in the rate of change of electrical conductivity ((dG/dt)1) (Figure 4) corresponding to the start of accumulation of lactic acid during of lactic acid fermentation.

After the intensive acidification process, a moderate acidification step, which starts in the range of 160 ÷ 170 min (pH = 5.3) follows the second conductivity minimum (Figure 4). From this moment to the end of the studied process, the process was monitored evenly in terms of lactic acid accumulation and conductivity.

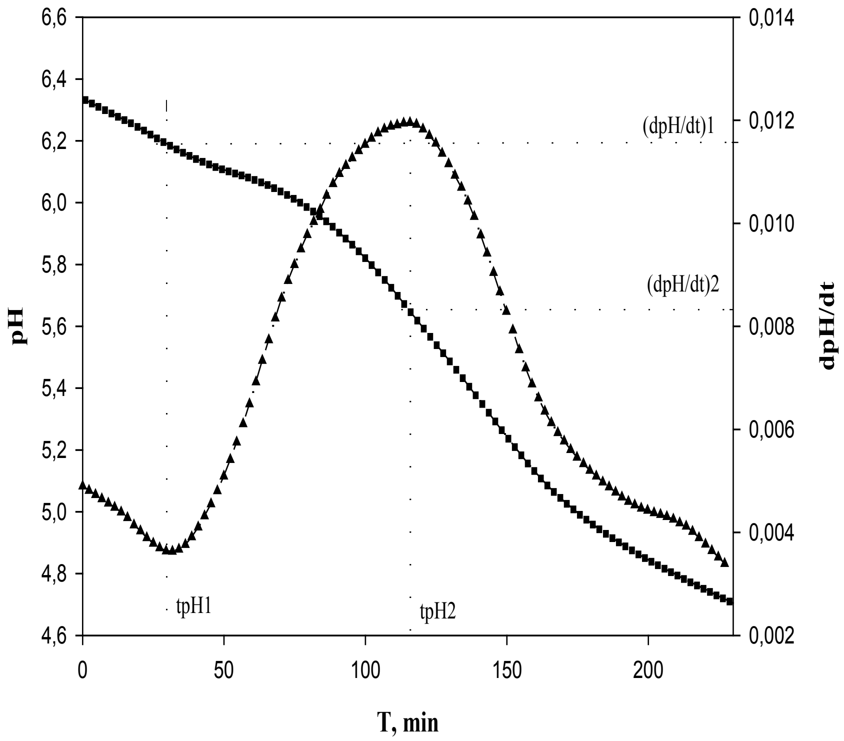

Figure 3. Change in pH of milk (■) inoculated with LAT BY 1-8 and the first derivative (dpH/dt) (▲) as a function of time.

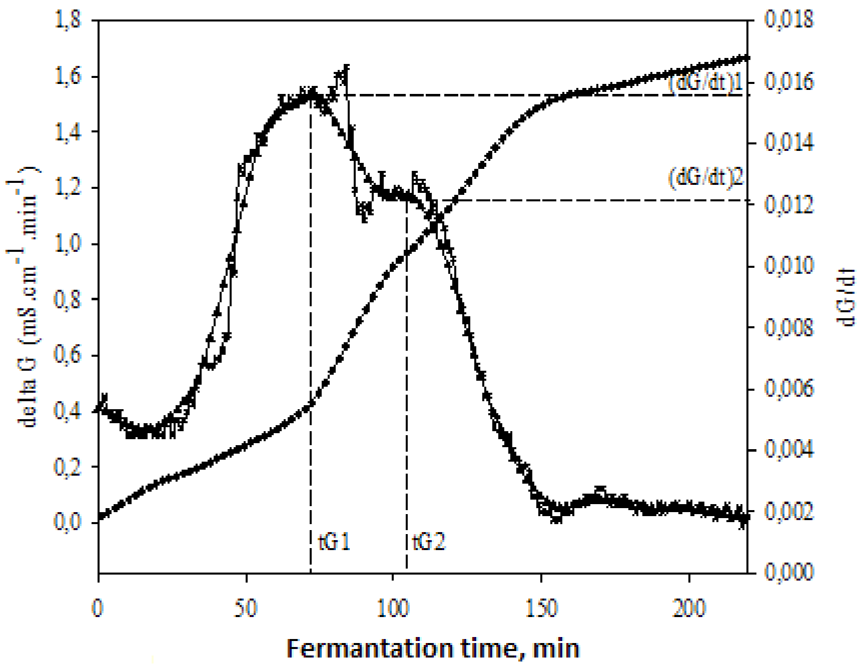

Figure 4. Changes in electrical conductivity (Δ G) (●) during acidic coagulation of milk inoculated and the first derivative (dG/dt) (▲) as a function of time.

The mathematical model of the data describing the change in conductivity during acid coagulation, was performed by non-linear regression (Figure 5). From the normality test it was concluded that the data are normally distributed ($W = 0.9600$) at a level of significance $p < 0.05$, which allows to continue statistical procedure.

The results of the statistical analysis, including regression coefficients (R) of 0.9993, are an indicator of the correct description of the experimental data from the selected function.

The function is a sigmoid with three parameters: a, b and x0.

f = a/(1 + exp(-(x-x0)/b))

The pattern of change in conductivity during acid coagulation with LAT BY 1-8 has the following form:

f = 1.5281/(1 + exp(-(x-109.1578)/29.2098))

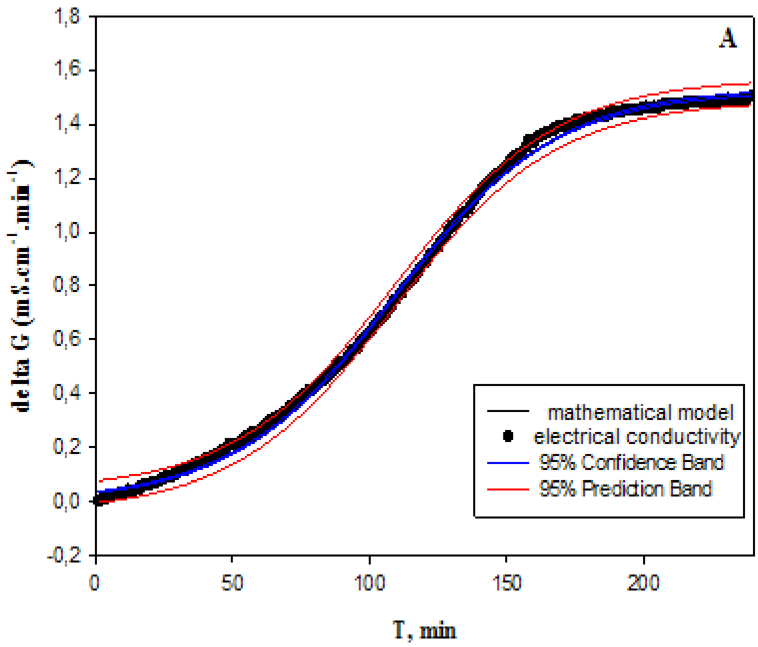

Figure 5. Regression model of the change of electrical conductivity during the acid coagulation.

The analysis of the results of the study of the change of conductivity in the course of the acid gel production showed a similar mechanism in the rate of lactic acid accumulation. Significant inflection points were found at close pH values, confirming the turbidimetry evaluation of acid coagulation.

CONCLUDING REMARKS

In consonance with the physicochemical changes established during acid gel formation, three pH ranges could be differentiated by the turbidimetric and conductometric methods.

A moderate acidification rate was demonstrated by an analysis of pH change and conductivity results in the range of pH = 6.0 ÷ 6.2 to 5.1 ÷ 5.2.

The analysis of the results of the study of the change of conductivity in the course of the acid gel production showed a similar mechanism in the rate of lactic acid accumulation. Significant inflection points were found at close pH values, confirming the turbidimetry evaluation of acid coagulation.

The resulting mathematical model by sigmoid function with three parameters can be used in future research to predict a change in electrical conductivity during acid coagulation.

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НЕДЕСТРУКТИВНА СТУДИЈА НА КОАГУЛАЦИЈА НА КИСЕЛО МЛЕКО

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Резиме

Коагулацијата на киселина е главниот чекор на производство на јогуртот и има големо влијание врз текстурата на јогурт, микроструктурата и реологијата, придонесувајќи за целокупната сензорна и визуелна перцепција на потрошувачот. Целта на овој труд беше да се проучат различните фази на формирање на киселински гел со турбидометриски и кондукционометриски методи. Недеструктивните методи кои се користат овозможуваат диференцијација на три региони: латентна фаза (rN = 6,3 ÷ 6,0), експоненцијална фаза (rN = 5,9 ÷ 5,4) и фаза на формирање на протеинска структура на деминерализирани казеински мицелии (pH = 5.3 ÷ 4.6). Математичкиот модел добиен со трипараметарската сигмоидна функција може да се користи во идните студии за да се предвиди промената на спроводливоста за време на коагулацијата на киселини.

Клучни зборови: *турбидометриски метод, кондукционометриски методи, коагулација на киселина.*