Scientific Bulletin. Series F. Biotechnologies, Vol. XIX, 2015
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

MONITORING OF ENZYMATIC COAGULATION OF COW'S MILK AT LOW TEMPERATURES BY AN OPTICAL METHOD

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Abstract

The kinetics of enzymatic coagulation of cow's milk was monitored by automated optical device. The optical method used for analysis and control of the coagulation process is non-destructive, which creates prerequisites for accurate analysis and forecasting process together with the applied numerical differentiation of the results obtained. The use of the optical device is determined by the relative change (%) of the intensity of light permeate flow during the process.
In cheese production, the process of the specific enzymatic coagulation of milk begins at temperatures below 20°C. Under these conditions, the coagulation occurs only in the enzymatic phase. In order to achieve the necessary energy levels to continue the process during the remaining phases, an increasing of the temperatures up to 35÷38°C was made.
The milk coagulants used in the experiment were: calf chymosin, microbial and camel enzyme, in quantities 30 $cm^3.10^{-2}.dm^{-3}$, with enzyme activity reduced to 1:10000.
Practical implementation of the optical method and device results in refinement of the technological process for production of cheeses, facilitating the examination and the control of the process of enzymatic coagulation, leading to improved quality and typification of the final product.

Key words*: optical method, enzymatic coagulation, milk-clotting enzymes, automatic control, relative absorbance.*

INTRODUCTION

The enzymatic coagulation of milk is a basic operation in the production of cheese. The temperature used to conduct the coagulation affects the rate of the process. At a temperature of coagulation below 10°C and over 65°C coagulation is not observed. In the temperature range of 10÷20°C, the rate of the coagulation is reduced, over 20°C it increases progressively, until temperatures of 40÷42°C – the optimal temperature for the used milk-clotting enzymes in cheese manufacturing (Baltadzieva, 1996).
The temperature has less impact on the enzymatic phase of the coagulation and significantly affects the course of the aggregation phase. The abovementioned trend was confirmed by the Stenne – Hutin process, called "coagulation at low temperatures" (Szadkovska, 1978), investigated also by Berridge (Berridge, 1952).
At temperatures between 20°C and 40°C, the dissociation constant of the enzyme-substrate complex vary slightly, until the rate of aggregation of the casein micelles is doubled (Lagaude et al., 2004).
When milk coagulants are placed in milk at 5°C, coagulation does not occur. After temperature increase a coagulum is formed faster than in the case of normal coagulation conditions. It was proved that at a low temperature only the enzymatic phase of hydrolysis of k-casein fraction of the casein micelles takes place. Therefore the casein micelles lose their stability and an increase of temperatures results in accelerated aggregation and faster rate of gelation (Dalgleish, 2009).
Morgenroth (Morgenroth, 1899) established that at low positive temperatures coagulation was not observed in milk. When the milk-clotting enzymes were left several hours in the mentioned conditions and then the milk was heated, it coagulates in a very short time.
Berridge (Berridge, 1952) has given a biochemical explanation of the processes occurring during "cold coagulation" - in the first stage of the coagulation, at low

temperature, part of the molecule of the k-casein fraction of the micelles was released, resulting in loss of its stabilizing properties. In the second stage begins general destabilization and aggregation of casein micelles. Further heating causes instant milk coagulation.

Based on Beridzh and Stenne – Hutin studies, methods and a technological equipment for the implementation of the continuous coagulation of milk were developed (coagulation in stream).

Different instruments and devices were developed for implementation of continuous coagulation of milk, based on the separation of enzyme and agregation phase - systems NIZO (the coagulation in stream covers the processes of coagulation up to placement in molds) and Sten-Hautin (two-stage coagulation using condensed milk concentrated to a ration 3:1) (Ramet, 1980).

Monitoring of the phases of the coagulation process is conducted using various methods and devices, differing in principle of action and constructive characteristics.

Dynamic and static instrumental techniques are mainly used, such as heuristics, rheological methods, "the hot wire method", ultrasonic methods, optical methods, microscopic methods and others. (Lucey, 2002; Hassan, 1995; Anderson, 2003).

The practical application of optical methods and systems leads to an optimization of the technological process in the production of cheeses, allowing precise analysis of the individual phases of enzymatic coagulation, thereby increasing the yield and improving the quality characteristics of the obtained finished product.

The aim of the conducted study was to monitor the enzymatic coagulation of cow milk, at low temperatures, using an automatic optical device.

MATERIALS AND METHODS

For the purposes of the experiment was used raw cow milk, obtained from the farm of the University of Agriculture, Plovdiv, cooled to 4 °C during the transportation, in order to preserve its quality characteristics.

The determination of the physicochemical parameters of the cow milk used for the experiments was performed by an ultrasonic milk analyzer. The values obtained are the following: protein – 3,36 %; fat – 4,9 %; non-fat solids – 8,75 %; density – 1,029 $g \cdot cm^{-3}$; titratable acidity – 18 °T; pH – 6,7.

Enzymes of different origins were used as milk coagulants - calf chymosin, with activity 1: 10000, enzyme of camel origin, strength 1: 70000 and microbial milk-clotting enzyme with activity 1:50000, produced and delivered by Cr. Hansen Company. To conduct the experiments, the enzyme activity was adjusted to 1:10000.

The amounts of the milk-clotting enzymes were 40 $cm^3.10^{-2}dm^{-3}$.

The monitoring of the process of rennet coagulation was performed by automatic optical device with an online application. Experimental data is archived by a system based on Data Loggers and software for their visualization by major information network - filing system MS DL 3.02.

The elements (optical fibers) of the optical device have a diameter d = 5 mm. The main beam of light was realized by a source with a wavelength corresponding to the absorption of the gel-forming structures (protein components) at 600 nm.

The process of coagulation at low temperatures was implemented using the following temperature values: 5, 10, 15, 20, and 25°C. Four multiple repetitions of the experiment were performed. For the statistical and mathematical analysis were used the average values obtained from the various experiences.

The volume of the samples was 500 $cm^3.10^{-2}dm^{-3}$.

The obtained experimental data were processed statistically by specialized mathematical software - Sigma Plot 11.0.

RESULTS AND DISCUSSIONS

An experiment was conducted to identify the amendment of the phases of enzymatic coagulation of cow milk, depending on the tested temperatures of coagulation, using an optical system.

An important element of the system is the optical part illustrated in Fig. 1.

The module of the light system is composed of a flexible optical fiber, firm optical fiber and a tip, presented in Fig. 2. The optical fibers end

with plugs, allowing connection to sensors and light source.

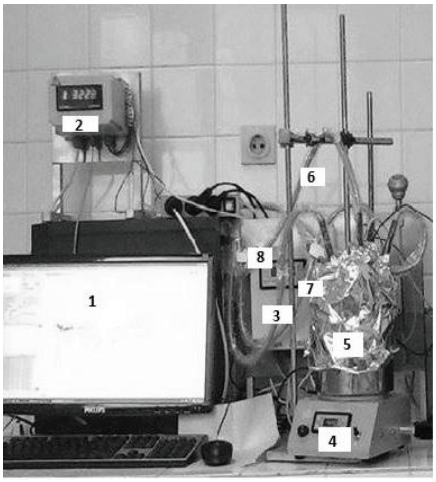

Figure 1. System for monitoring the coagulation of milk (1 – PC; 2 – electronic block of the optical device; 3 – device for measuring temperature; 4 – water bath with electronic thermostat; 5 – milk sample; 6, 7, 8 – optical fibers of the device)

The hardware of the optical laboratory device for monitoring the rennet coagulation is shown in Fig. 3.

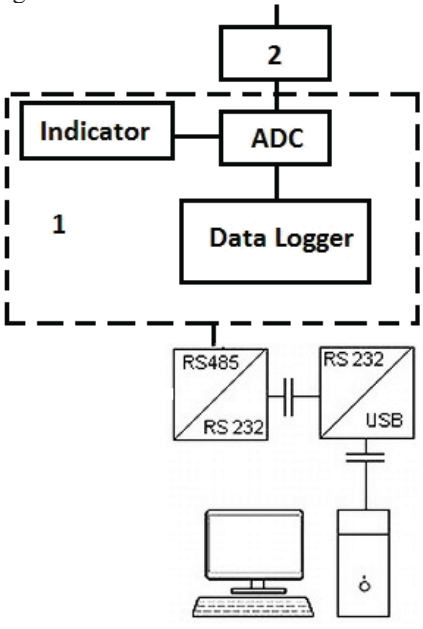

Figure 3. 1 – ADC, indicator and datalogger; 2 – compensating module; 3 – PC connection (RS 232)

The system can monitor the variation of the reflected and scattered light. The relative optical density (ROD, %) in the environment is monitored by establishing the variation of the intensity of the main beam (I_0), formed from a source of light in the visible part of the spectrum, and that of transmitted light (I_1).

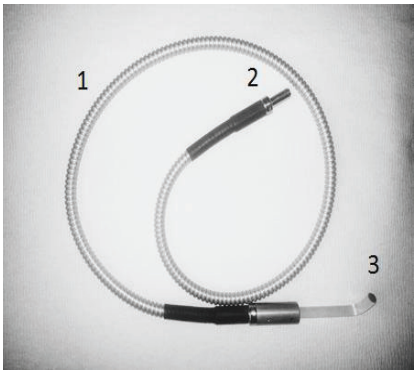

Figure 2. 1 – flexible optical fiber; 2 – tip; 3 – firm optical fiber

The influence of temperature on the course of enzymatic and aggregation phases of rennet coagulation was established and determined by the optical system of automatic control.

The temperature range investigated in the experiment include temperatures between 5÷25°C (considered as low temperatures of coagulation because the optimal temperature for conducting the process is 35÷37°C).

At each of these temperatures in the milk was added milk-clotting enzyme, then the sample was retained for a period of 30 min, followed by heating to a temperature of coagulation (35÷37°C). The duration of each of the conducted trials was 90 min.

The results obtained using calf chymosin are presented in Fig. 4.

A decrease in the values of the relative optical density was observed at temperatures 5÷15°C before heating the samples, which correlates with the theoretical statements of Stenne – Hutin and Berridge (Berridge, 1952; Szadkovska, 1978).

The negative values of the relative optical density are associated with the enzymatic phase of the coagulation process, related to the beginning of the hydrolysis of the k-casein

fraction of the protein micelle and the separation of glycomacropeptide residue. This reaction determines the destabilization of the protein micelle and the course of the enzymatic phase of the coagulation process.

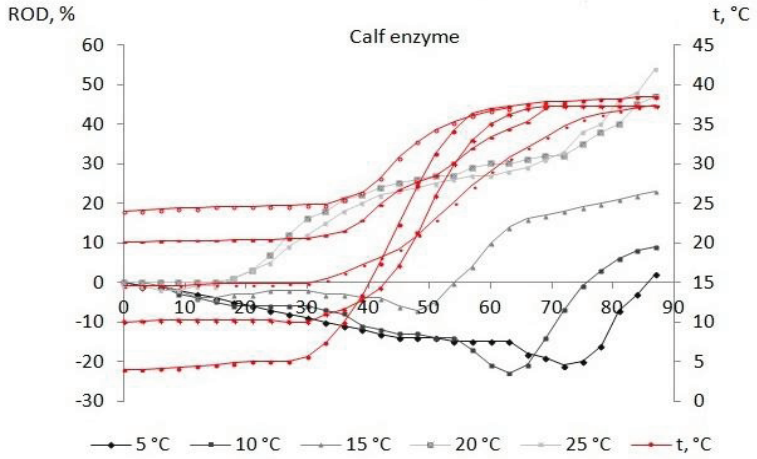

Figure 4. Monitoring of the enzymatic and aggregation phases of the coagulation process, using calf chymosin

The results presented in Figure 4 show that at temperatures 5÷15°C prevalence of enzymatic phase to that of aggregation is recorded, evidenced by the values of ROD, % remained negative until the start of heating. Increasing the temperature to 35÷37°C, the aggregation phase of the enzymatic coagulation begins.

As the temperature increases up to 35÷37°C (the optimal temperature for the coagulation process), the values of the relative optical density become positive, due to the preponderance of the aggregation phase and the flocculation reaction.

It was observed that in the temperature interval 5÷15°C, the enzymatic phase was prolonged depending on the temperature of the retention - at 5 °C the beginning of the aggregation phase was observed after reaching the temperature of coagulation, and by heating for a period of 25 min. The trend was similar for 10 and 15°C. Differences were observed in the aggregation time – for the first sample (10°C) the heating period is 10 min, while for the second (15°C) the aggregation phase occurs upon reaching 35°C.

Acceleration of the aggregation phase at temperatures of 20÷25°C was reported. The values of the relative optical density are positive, even before the heating starts i.e. the aggregation phase occurs before the optimal temperature of coagulation was reached.

Using bovine chymosin, intensive flocculation and increase of the degree of aggregation was observed, at a temperature of 16 ÷ 17°C.

Analogous experiment was conducted using an enzyme of camel origin. The results are presented in Fig.5.

The results presented on Figure 5, show similar trend, observed when calf chymosin was used.

At temperatures 5÷15°C prevalence of enzymatic phase to that of aggregation was marked, evidenced by the negative values of the relative optic density until the start of heating.

Differences, between the two enzymes (calf and camel), related to the aggregation time were noticed in the temperature interval 5÷15 °C, - when the camel enzyme was used, at 5°C and 10 °C, the same heating time was needed for the aggregation phase to start, after increasing the temperature to 37°C – approximately 25 min.

The aggregation phase in the sample kept at 15 °C occurs upon reaching 35°C, the same trend observed when the calf chymosin was used.

Reaching temperatures of 17÷18°C, an accelerated rate of aggregation by registering preponderance of the flocculation phase was established for the entire temperature range investigated.

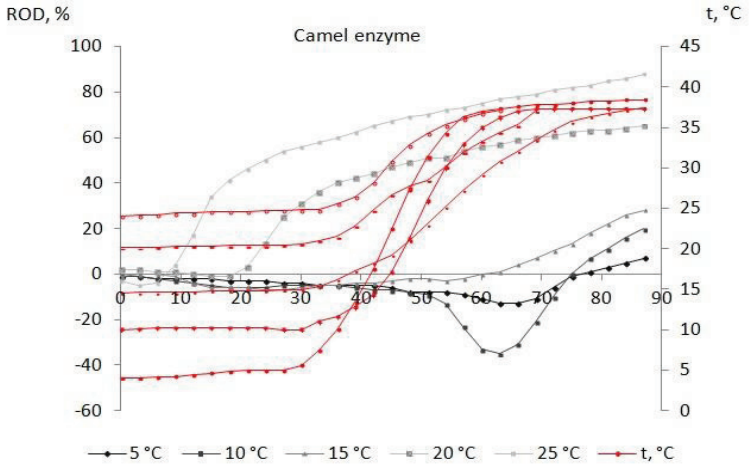

Figure 5. Monitoring of the enzymatic and aggregation phases of the coagulation process, using camel coagulant

The results obtained using an enzyme of microbial origin are presented in Fig. 6.

The results when the microbial enzyme was used differ significantly from the data obtained for the other two milk-clotting enzymes.

At temperatures 5÷15°C, lowest values of the relative optical density were reported before heating the samples, reaching – 50 relative optical units. At temperatures of 5°C the enzymatic phase was observed by heating to temperatures of coagulation for a period of 25 min, as in the case of the other two tested enzymes. Differences were established in relation to the aggregation phase - the flocculation was not observed by increasing the temperatures to 37°C, and the values of the optical density were negative by the end of the experiment. The trend observed for the samples kept in 10 and 15°C was similar to the other two examined coagulants.

Increasing of relative optical density values were recorded at a temperature of 25°C, associated with an increase in the rate of aggregation of casein structures and a formation of flocculates.

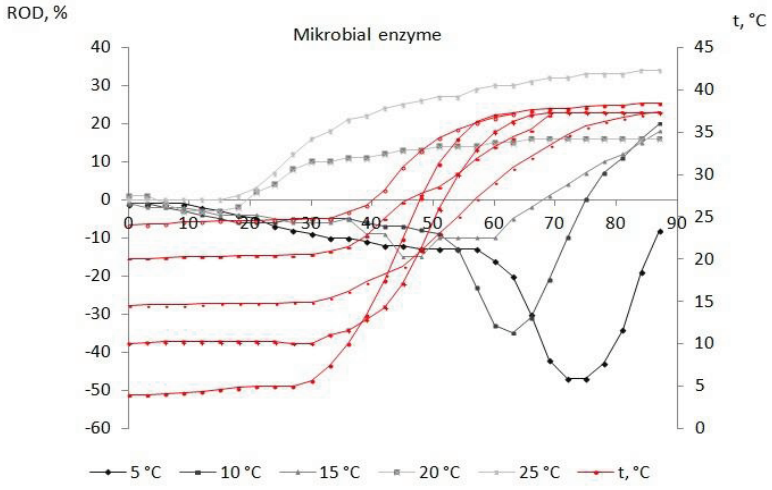

Figure 6. Monitoring of the enzymatic and aggregation phases of the coagulation process, using microbial coagulant

Depending on the type of enzymes and the values of the coagulation temperature, the phases of the coagulation process occurring at a different pace and with different characteristics. For all three milk coagulants, the enzymatic phase of the coagulation process was observed at low temperatures. However, using calf chymosin, the enzymatic phase was started without increasing the temperature and before heating.

Similar trend was established for the other two tested milk coagulants, different from the one observed for the calf chymosin: the course of the enzymatic phase and the decrease of the relative optical density are registered at increased temperatures, respectively 18÷20°C. These conditions provide the energy required for the implementation of the hydrolysis reaction of the k-casein.

However, when the calf chymosin was used, the temperature significantly affect the duration of the enzymatic stage, the time for aggregation and the rate of the flocculation of the protein component. The rate of aggregation is maximum when the sample was heated to a temperature of 20÷25°C, as similar trend was established when microbial enzyme was used. The fastest rate of the flocculation was observed when enzyme of camel origin was tested - aggregation was observed by increasing the temperatures to 17÷18°C, i.e minimum amount of energy was required for the beginning of the flocculation.

The results obtained using milk-clotting enzyme of camel origin are related to the action and characteristics of the enzyme - strong specificity for the k-casein fraction of the milk, resulting in accelerated aggregation and flocculation phase without significant influence of the temperature on the rate of the phases of the coagulation process.

CONCLUSIONS

The results of the conducted experiments establish the characteristics of the enzymatic and aggregation phases of the enzymatic coagulation, using three different genetic variants of milk-clotting enzymes.

Significant factor in the progress of enzymatic and aggregation phase of the coagulation process is the temperature of coagulation of milk. Low positive temperatures of the coagulation (5÷15°C) have a slight influence on the enzymatic phase and are significant factor in flocculation and aggregation phase - the acceleration of the aggregation phase was observed with increasing temperature for the three tested milk-clotting enzymes.

The developed automatic optical system used in the experiment can be applied in manufacturing practice for examination of the dynamics of the technological processes in the production of cheese, optimization and control of the coagulation process with the necessary practical accuracy.

The obtained results and dependencies are applicable in cheese production according to the conditions and types of enzymes used. They can be applied for a stream dosage of the coagulants, regulation of syneresis and preparation of technological schemes for the production of various types of cheeses.

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