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# STUDY OF THE STRAIN OF DISPLACEMENT VARIATION DURING THE COMPACTION OF RENNET GEL OBTAINED BY VARIOUS MILK-CLOTTING ENZYMES

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***Abstract***

*The variation in the rate of formation of rennet gel depending on the use of different types of milk-clotting enzymes for a set time was studied.*
*The intensity of development of the phases for the research process was determinate by the specificity and activity of coagulants. It was found that the strain of displacement was directly related with the time for the gel formation and also with the types of rennet enzymes used for its preparation.*
*The study was conducted with the amounts of rennet enzyme from 10 to 60 $cm^3$ $x10^{-2}dm^{-3}$ and the strain of displacement of the gel changes as values for a period of 90 min. The data obtained from the measuring instrument for the strain of displacement using milk-clotting enzyme with camel origin was in the range of $7,2x.10^{-6} \div 1,3x10^{-5}$. It was determined that under the similar conditions after 30 min there were no significant changes, these results demonstrate the ability of this enzyme to finalize the process more quickly. Using the enzyme of microbial origin, the values derived for the strain of displacement are $\Theta = 3,4x10^{-6}$ after 30 min of the experiment and $\Theta = 1,20x10^{-5}$ at the end of the conducted experiment. The use of this milk coagulant indicates significant changes in the strain of displacement at the initial stage of formation of the gel. It was recorded that differences were reduced as the experiment progresses.*
*The data obtained from the penetrometric study were processed statistically and the results can be apply in laboratory and manufacturing practices using the chymosin of microbial origin and camel chymosin obtained by fermentation.*

***Keywords:*** *rennet coagulation, penetrometry experiment, milk-clotting enzymes*

## INTRODUCTION

The first stage of cheese manufacture is the conversion of liquid milk to cheese curd. This process is achieved by the addition of rennet to coagulate the milk and by the subsequent expulsion of the whey by syneresis (Home, 2004; Banks, 2004).
Rennet is the generic name for an enzyme preparation used to coagulate milk in the production of rennet coagulated cheese and rennet casein. The principal role of the enzymes in rennet is to coagulate milk, but they also contribute to proteolysis during the ripening of most cheeses (Bansal et al., 2009; Valkova-Jorgova, 2005; Danov, 2009).
The increase in cheese production, coupled to a diminishing supply of natural animal rennet, is responsible for increases in the demand for alternative milk-coagulating sources. Due to this and a variety of outher factors attention is being turned to the use of microbial coagulants (Garcia et al., 2012).
With developments in recombinant DNA technology, the gene for calf chymosin has been cloned into microorganisms, permitting the production of chymosin by fermentation. Such ‘‘fermentation produced chymosins’’ are now widely used for cheese manufacture in

many countries and give excellent results (Ernstrom and Wong, 1974; Fox and McSweeney, 1997; Green, 1977; Nelson, 1975; Phelan, 1985; Sardinas, 1972; Sternberg, 1976). For milk-clotting enzymes other than calf rennet, curd firmness and syneresis ideally should be similar to those when calf rennet is used, and there should be no significant losses of fat or protein. Actual rate of curd firming may not be important for determining properties of the curd (Green, 1977) but it's control is important in cheese making (Yun, 1982). Variations of curd firmness at time of cutting may result in greater losses of milk components and reduced cheese yield (Olson, 1982). Monitoring curd firmness during cheese making offers the potential for reducing such losses by cutting at consistent curd firmness to optimize cheese manufacturing (Olson, 1982). Rate of increase of curd firmness is reduced as the extent of proteolysis of casein increases (Yun, 1981). Excess proteolysis apparently inhibits curd firming by degrading the protein molecules involved in curd formation (Yun, 1982). Some methods of monitoring curd firmess include: vibrating reed viscometer (Marshall, 1982), pressure transmission systems (PTS) (Garnot, 1982; Marshall, 1982; Vanderheiden, 1976), Formagraph (McMahon and Brown, 1982; McMahon and Brown, 1983; Reuter et al., 1981), and resistance to penetration or cutting of the curd (Ritchardson et al., 1971; Storry and Ford, 1982).

## MATERIALS AND METHODS

In order to clarify the influence of the type of milk-clotting enzymes on the firmness of rennet-obtained gels, penetrometric study was conducted with the use of coagulated cow's milk.

The physicochemical parameters of the cow's milk were: Fat – 3,6 % (after degreasing – 2,76 %); Non-fat solids – 8,4 %; Density of milk – 1,027 kgxdm$^{-3}$; Protein – 3,11 %; Acidity – 16; Active acidity (pH) – 6,8.

Before processing, the milk was subjected to a partial degreasing to reduce the effect of milk fat on the obtained results, and was heated to temperatures of 65 ° C.

For a period of 90 min, measurements were made every 10 min, and the duration of the measurements was 60 s.

As coagulant was used camel chymosin of „Cr. Hansen", under the name "CHY – MAX" ® M, with milk-clotting activity 1000 IMCU / ml (or enzymatic activity 1:70000), stored refrigerated at 0 ÷ 8 °C.

The milk-clotting enzyme of microbial origin, produced by "Cr. Hansen", was described as activity (1:50000) and other indicators in established certificate. For the experiment, the enzymatic activity of the aforementioned coagulants was reduced to 1:50000.

For determination of the density of the rennet-obtained gel was used a type of penetrometer, appliance developed by Todor Todorov Lyubenov, UFT, Plovdiv (Lyubenov, 1975). The device has a working body with cylindrical shape and a flat work surface area with F = 2x10$^{-4}$ m$^2$, 0,0139 kg mass and constant k = 0,5 Nxkg$^{-1}$. The strain of displacement Θ was calculated by moving the operating body (h).

$\Theta = k.m.h^{-2} = 0{,}00695xh^{-2}$, Nxm$^{-2}$

All results were statistically interpretated to a level of significance of $\alpha = 0{,}5$. Data were statistically processed using specialized mathematical software - OriginPro 6.1.

## RESULTS AND DISCUSSIONS

The aim of the conducted experiment was the examination of the process of rennet gel formation using different types and amounts of milk-clotting enzymes, also called chymosin or rennin. The rate of gel formation for a set time, with three different genetic variants of enzymes was studied. The experiment was focused on the enzymes of camel and microbial origin, compared with calf chymosin. It was found that the strain of displacement of the rennet obtained gel was related with the gel formation time and the specific activity of the coagulants used in the experiment.

Penetrometric methods were used for the gel hardness and firmness determination. The results of the penetration determination were obtained in mm. The data obtained from the analysis for the three different types of milk-clotting enzymes are presented in Table 1. The results demonstrate variations of the values for

a period of 10 to 90 min with the amounts of rennet enzyme from 10 to 60 $cm^3x10^{-2}$ x $dm^{-3}$. The amount of rennet for the specific experiment was taken as a constant value (30 $cm^3x10^{-2}xdm^{-3}$) for the three milk-clotting enzymes used for the preparation of the rennet gel.

Table 1.Penetration of coagulum obtained using milk-clotting enzymes with microbial, camel and calf origin

| Penetration time, min | Penetration (mm) in the rennet- gel, obtained using milk-clotting enzymes, (30 $cm^3x10^{-2}x$ $dm^{-3}$ ) for 60 s. | | |
|---|---|---|---|
| | Microbial chymosin | Calf chymosin | Camel chymosin |
| 10 | 57,2 ± 5,1 | 32,0 ± 2,8 | 38,0 ± 3,1 |
| 20 | 47,1 ± 3,8 | 29,2 ± 2,3 | 31,6 ± 2,5 |
| 30 | 42,3 ± 3,4 | 29,0 ± 1,9 | 29,2 ± 1,8 |
| 40 | 35,5 ± 2,2 | 28,2 ± 2,1 | 28,6 ± 2,4 |
| 50 | 31,3 ± 2,6 | 28,1 ± 2,2 | 26,1 ± 1,8 |
| 60 | 29,0 ± 2,8 | 27,6 ± 1,7 | 25,6 ± 2,2 |
| 70 | 27,6 ± 1,9 | 27,2 ± 2,3 | 25,2 ± 2,1 |
| 80 | 26,0 ± 2,6 | 26,4 ± 1,9 | 24,0 ± 2,0 |
| 90 | 24,1 ± 1,4 | 25,2 ± 2,1 | 23,0 ± 1,8 |

Table 2. Strain of displacement of coagulum, obtained using milk-clotting enzymes with microbial, camel and calf origin

| Time for penetration, min | Strain of displacement ($Nx.m^{-x2}.10^{-6}$) in rennet-gel, produced using milk-clotting enzymes, in an amount of 30 $cm^3.10^{-2}.dm^{-3}$ for 60 s. | | |
|---|---|---|---|
| | Microbial chymosin | Calf chymosin | Camel chymosin |
| 10 | 2,12 ± 0,14 | 6,79 ± 0,11 | 4,81 ± 0,16 |
| 20 | 3,13 ± 0,11 | 8,15 ± 0,14 | 6,96 ± 0,16 |
| 30 | 3,88 ± 0,12 | 8,26 ± 0,18 | 8,15 ± 0,17 |
| 40 | 5,51 ± 0,18 | 8,74 ± 0,23 | 8,50 ± 0,24 |
| 50 | 7,09 ± 0,16 | 8,80 ± 0,41 | 10,20 ± 0,34 |
| 60 | 8,26 ± 0,24 | 9,12 ± 0,34 | 10,60 ± 0,43 |
| 70 | 9,12 ± 0,24 | 9,39 ± 0,42 | 10,94 ± 0,58 |
| 80 | 10,28 ± 0,66 | 9,97 ± 0,74 | 12,07 ± 0,62 |
| 90 | 12,87 ± 0,84 | 10,94 ± 0,64 | 13,13 ± 0,81 |

Using the data from Table 1 and after mathematical processing of the results presented, the average values of the strain of displacement are defined. The results for the strain of displacement for the tested enzymes are presented in Table 2.

The results demonstrate great variations between the values obtained for the enzyme with microbial origin compared with those obtained from the calf chymosin. The differences were explained through the mechanism of the gel forming: in the first 30 to 40 min the formation and the firming process of the gel produced with the microbial chymosin were very slow and the coagulum was too fragile and easy to be destroyed. The milk gel was enough tick and had the density needed for cutting at the end of the conducted experiment. The phases of coagulation process using this type of enzyme were delayed in time. The final values of the strain of displacement after 90 min of the experiment demonstrate the similarity in the results for these three different coagulants.

Typical for the milk-clotting enzyme with calf origin the strain of displacement had values almost similar during all the experiment, and the variations between the different periods of measurements were not significant.

The data between 30 and 60 min showed almost the same values for the strain of displacement i.e. using this type of milk-clotting enzyme the gel density reaches certain state and after that the hardness of the coagulum rest unchanged or varies slightly until the final phase of the rennet coagulation.

The results of the experiment using camel chymosin produced by fermentation demonstrate a faster rate of coagulation during the first two phases of the process and a relatively slower rate in the next phases of the process. The use of minimal amounts of the aforementioned coagulant delays the compaction of the rennet gel in comparison of the control chymosin. Using quantities of 30 $cm^3$ $x10^{-2}$ $xdm^3$, the strength of the rennet gel after 20 min was similar to that of the other two milk-clotting enzymes.

The values of the strain of displacement with the use of the coagulant of camel origin at the 90 min of the experiment are relatively equal and have no significant differences in comparison with the other two milk coagulants used to conduct the penetrometric study.

The mathematical and statistical processing leads to a nonlinear regression model, and the polynomial liner equation having the following form:

$$\mathbf{f = y_0 + a*x} \quad (1)$$

The mathematical models derived can be used in laboratory and manufacturing practice directly in the coagulation process consistent with the specific factors and the particular conditions.

Figures 1, 2 and 3 represent the mathematical modeling for the three types of the coagulants studied in the renneting process and the formation of the gels.

For the milk-clotting enzyme of camel origin the correlation coefficient R = 0,9930, the quadratic coefficient $R_{sqr}$ = 0,9861 obtained, and coefficients of significance of the aforementioned value shows that the mathematical model describes correctly the relation studied and the research process of milk coagulation. The mathematical model that characterizes the formation of the gel using chymosin of camel origin has the following form (Figure 1):

$$f = 0,4444 + 0,1295*x \quad (2)$$

Verification of the adequacy of the obtained mathematical model was made according to the tables in the confidence level α (0,5).

It was determined that under similar conditions after the 30 min there were no significant changes in the values obtained for the strain of displacement, which shows the ability of this enzyme to finalize the process more quickly in comparison with the other two enzymes that were used.

The experiment with the use of calf chymosin as a control was also conducted. The statistical processing was made analogous to the previous experiment. For the bovine chymosin the results shows variations of the strain of displacement in the confidence and prediction bands (intervals). Verification of the adequacy of the obtained mathematical model was made according to the tables in the confidence level α (0,5), and the correlation coefficient R = 0,9815, the quadratic coefficient $R_{sqr}$ = 0,9633 obtained, and coefficients of significance of the aforementioned values shows that the mathematical model describes correctly the examined process. The mathematical model that describes the investigated dependence with the use of bovine chymosin is in the form (Figure 2):

$$f = 4,7936 + 0,0938*x \quad (3)$$

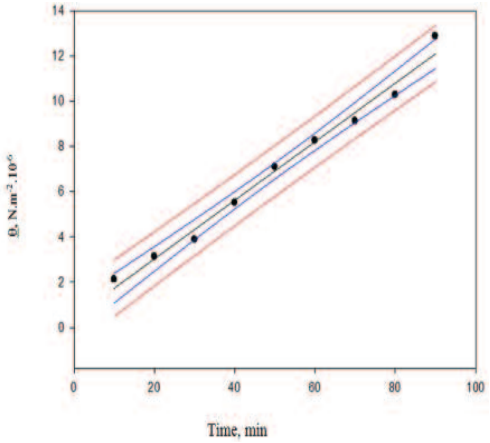


Figure 1. Variation of the strain of displacement in time using camel chymosin

| Coefficient | | Standard Error | t | P |
|---|---|---|---|---|
| $y_0$ | **0,4444** | **0,3273** | **1,3580** | **0,2166** |
| a | **0,1295** | **0,0058** | **22,2607** | **<0,0001** |

The mathematical model with the use of microbial chymosin was obtained following the same mathematical and statistical procedure as in the previous experiments and has the following form (Figure 3):

$$f = 6,8483 + 0,0412*x \quad (4)$$

According to the correlation coefficient R = 0,9591, $R_{sqr}$ = 0,9198 obtained and coefficients of significance of the aforementioned values, the mathematical model describes properly the process of milk coagulation with the use of microbial chymosin.

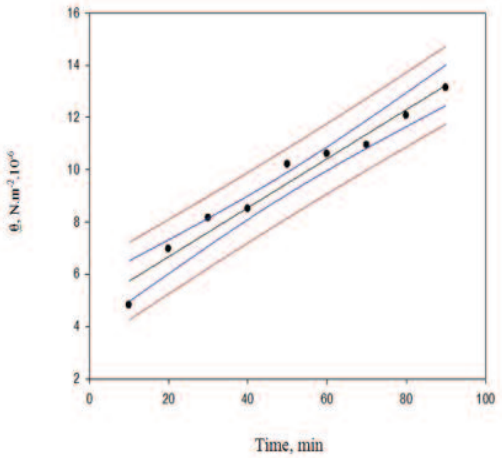


Figure 2. Variation of the strain of displacement in time using calf chymosin

| Coefficient | | Standard Error | t | P |
|---|---|---|---|---|
| $y_0$ | **4,7936** | **0,3897** | **12,3014** | **<0,0001** |
| a | **0,0938** | **0,0069** | **13,5479** | **<0,0001** |

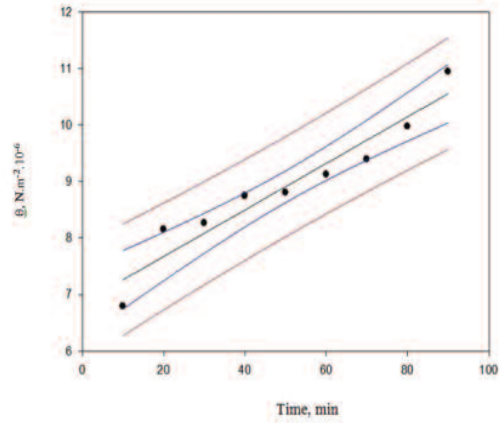


Figure 3. Variation of the strain of displacement in time using microbial chymosin

| Coefficient | | Standard Error | t | P |
|---|---|---|---|---|
| $y_0$ | **6,8483** | **0,2585** | **26,4932** | **<0,0001** |
| a | **0,0412** | **0,0046** | **8,9618** | **<0,0001** |

## CONCLUSIONS

The results of the conducted penetrometric studies indicate that the modifications of the structure and the formation of the rennet gel obtained by using milk-clotting enzyme with camel origin for a period of 90 min were with uniform compaction unlike the fast rate of gel compaction with the use of bovine chymosin and the slow one when microbial chymosin was used.

Based on the statistical analysis, in case of coagulation induced by calf chymosin, it can be concluded that there is no significant differences between the values of the strain of displacement in the period 30 - 50 min of the gel formation. The coagulation process can be divided in three phases: the first one is the period up to 20 min, second one is the stage between 20 and 50 min, and the final phase of the formation of coagulum is between 60 and 90 min.

The gel formation with the use of microbial coagulant presents statistically significant differences between the values of the strain of displacement during the entire coagulation process, excluding the data from 80 to 90 min. These results suggest smooth and uniform changes during the formation of the gel.

Compared with the rate of coagulation of the before mentioned coagulants, the course of gelation with the use of chymosin with camel origin is intermediate.

The three types of gels are formed for a period of 90 min. Between 80 and 90 min there are no significant variations in the values of the strain of displacement, thus it can be concluded that the three different types of gels have similar structural and mechanical properties.

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