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# STUDY OF THE EFFICIENCY OF THE METHOD USED FOR DETERMINATION OF THE STRAIN OF DISPLACEMENT OF RENNET GEL OBTAINED BY VARIOUS MILK CLOTTING ENZYMES

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***Abstract***

*During the penetrometric study, the efficiency of the method for determining the strain of displacement in rennet gel obtained by chymosin of microbial and camel origin was studied.*
*Using the standard measuring time of 60 s, the monitoring of penetration in the gel for the time of 30 s was also investigated. This approach is applied in defining the strain of displacement from the 10 to 90 min during the formation of the gel.*
*The results obtained by the penetrometric study of the rennet gel are the basis for modification of the method of determination of the strain of displacement. The serum released from the gel during the penetrometric study affects measurement accuracy.*
*The results obtained for 30 s indicate better stability of the experimenal data, which shortening the time for its implementation.*
*The information obtained by the conducted study suggests new modification of the method related to the time of penetration from the 60 to the 30 s.*

***Keywords****: penetrometric method, rennet coagulum, milk-clotting enzymes, gel firmness.*

## INTRODUCTION

Enzymatic milk coagulation for cheese manufacturing involves the cleavage of the scissile bond in k-casein by an acid protease. Bovine chymosin is strongly recommended enzyme, combining a strong clotting activity with a low general proteolytic activity (Valkova, 2005; Kappeler et al., 2006). Camel chymosin has different characteristics comparing with bovine chymosin. Camel chymosin demonstrate a 70% higher clotting activity than bovine milk and has only 20% of the unspecific protease activity of bovine chymosin. The camel chymosin, obtained by fermentation, is more thermostable than bovine chymosin (Kappeler et al., 2006).
Rates of increases in rigidity during the first half of gel assembly were increased by increases in rennet concentration but maximum gel rigidity was not strongly affected. Curd firming rates vary from enzyme to enzyme with the more specific enzymes producing a firm gel more quickly (Kowalchyk, 1978).

The amount of rennet added to the milk has a large effect on the rate of the overall process. An increased rennet concentration leads to a shorter flocculation time, gel firmness starts to increase earlier and the rate of increasing is higher (Zoon et al., 1988; Hyldig, 1993; Lomholt & Qvist, 1997). While the gels are at the same stage in gel formation with respect to gel firmness, and the enzymatic reaction is completed, other differences between the gels must be responsible for the differences in gel firming rate. In addition, while rennet concentration seems to affect the structure of initial aggregates, it seems reasonable to expect that structural differences between gels, made with different rennet concentrations, can explain this effect (Law and Tamime, 2010). Rate of curd firming is not important for determining properties of the curd (Green, 1982), but its 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.

Most fundamental studies on the gelation process involved use of instruments developed for the continuous monitoring of curd firmness. However, for the testing of a large number of milks, continuous monitoring is too time consuming and a single measurement of curd firmness at a given stage during coagulation is used as a basis for comparison. One of the most suitable equipment for this type of test is a penetrometer. Penetrometres generally measure the deformation of the coagulum under a compressive load. This deformation is then inversely related to the firmness of the coagulum (Burgess, 1978). The firmness of the gel increases for several hours after gel formation, depending on the conditions. Rennet gels show linear viscoelastic behaviour, i.e. deformation is proportional to applied stress, for relative deformations up to 0.026–0.05 Pa (van Dijk, 1982; Dejmek, 1987; Hyldig, 1993). At larger deformations the gel structure will be damaged.

Most detailed rheological investigations of renneting has been carried out on skimmed milk, but Storry et al. (1983) and Grandison et al. (1984) did not find any effect of fat content on the coagulation time or gel strength of unhomogenised milks.

The aim of this study is to identify the efficiency of the method for determination of the strain of displacement of rennet gel.

Penetrometric study was conducted with the use of coagulated cow's milk in order to identify the influence of the type of milk-clotting enzymes on the hardness of rennet-obtained gels.

## MATERIALS AND METHODS

The milk used in this experiment had the following physicochemical parameters: Fat – 3,6 % (after degreasing – 2,76 %); Non-fat solids – 8,4 %; Density of milk – 1,027 kgxdm$^{-3}$; Protein – 3,11 %. These parameters were obtained using "Ekomilk" apparatus (Plovdiv, Bulgaria). Acidity was determined by titration – 16 °T; Active acidity (pH) – 6,8.

Partial skimmed milk was heated to temperatures of 65 ° C.

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

The milk-clotting enzyme of microbial origin (Cr. Hansen) was characterized by activity (1:50000) and other indicators in established certificate.

For the experiment, the enzyme 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).

$\Theta = kxmxh^{-2} = 0{,}00695xh^{-2}$, Nxm$^{-2}$

All results were statistically processed to a level of significance of $\alpha = 0{,}05$. Statistical processing of the data obtained in the course of the experiment was carried out using specialized mathematical software - OriginPro 6.1.

## RESULTS AND DISCUSSIONS

The aim of the conducted experiment was the examination of the efficiency of the method for determination of strain of displacement during the rennet gel formation with three different types of milk-clotting enzymes. The focus of the experiment was related to enzymes of camel and microbial origin, compared with calf chymosin. It was found that the strain of displacement of the rennet gel obtained was related with the time for the gel formation and the specific activity of the milk-clotting coagulants used.

The gel hardness and thickness were defined using penetrometric methods. The results for the penetration of the working body of the device applied in the rennet gel were obtained in mm. The amount of rennet for the specific experiment was taken as 30 $cm^3x10^{-2}xdm^{-3}$ for the three milk-clotting enzymes used for the preparation of the rennet gel.

The results obtained from the penetrometric study with the use of the three milk-clotting enzymes are presented in Table 1.

After mathematical and statistical processing was established that the values obtained for the penetration of the working body of the device after 60 min, for the interval of 30 s and 60 s related to the enzyme of microbial origin were statistically insignificant. For the milk-clotting enzyme of calf origin, this tendency was observed 50 min after the initial coagulation for both times of measurements; and finally, in the case of using the enzyme of camel origin – after 20 min for the same examinated times (30 s and 60 s).

This statistical insignificance of the values for the three enzymes applied can be explained by the uniform rate of increasing rigidity of the rennet gel.

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

| Penetration time, min | Penetration (mm) of coagulum, obtained by milk-clotting enzyme in an amount, 30 $cm^3$ x $10^{-2}$ x $dm^{-3}$ from: | | | | | |
|---|---|---|---|---|---|---|
| | Microbial origin, s | | Calf origin, s | | Camel origin, s | |
| | 30 | 60 | 30 | 60 | 30 | 60 |
| 10 | 45,1 ± 1,6 | 55,2 ± 5,1 | 30,7 ± 2,0 | 34,2 ± 2,8 | 37,4 ± 1,2 | 39,6 ± 2,1 |
| 20 | 38,2 ± 1,4 | 44,1 ± 3,2 | 26,8 ± 1,2 | 31,0 ± 2,3 | 33,2 ± 1,1 | 35,2 ± 2,5 |
| 30 | 33,4 ± 1,6 | 41,3 ± 3,1 | 26,2 ± 0,8 | 29,0 ± 1,8 | 29,8 ± 1,8 | 30,1 ± 2,8 |
| 40 | 28,2 ± 1,3 | 36,5 ± 2,4 | 25,4 ± 1,0 | 28,2 ± 2,0 | 24,6 ± 1,1 | 27,0 ± 2,6 |
| 50 | 27,3 ± 1,5 | 32,3 ± 2,6 | 25,0 ± 1,1 | 27,4 ± 2,4 | 23,4 ± 1,4 | 25,0 ± 1,8 |
| 60 | 26,0 ± 1,0 | 30,0 ± 2,8 | 24,1 ± 1,1 | 27,0 ± 2,0 | 22,2 ± 1,6 | 23,2 ± 2,2 |
| 70 | 25,0 ± 1,1 | 26,4 ± 1,9 | 23,2 ± 1,4 | 26,2 ± 2,3 | 22,1 ± 1,8 | 23,0 ± 2,4 |
| 80 | 24,2 ± 1,2 | 25,3 ± 2,4 | 21,2 ± 1,2 | 25,1 ± 2,0 | 19,0 ± 1,8 | 22,0 ± 2,1 |
| 90 | 22,2 ± 1,2 | 23,4 ± 1,5 | 20,0 ± 1,0 | 23,0 ± 2,4 | 17,1 ± 1,4 | 21,0 ± 1,8 |

Whereas, observing the results obtained for the both times of measurement, it was defined that the values of intrusion in depth in the rennet gel for a period of 60 s using all three types of enzymes were higher in comparison with the values for a time of 30 s.
The strain of displacement is directly related to the amount of the lactoserum separated of the rennet gel, i.e. the duration of the measurements define the amount of the serum released from the rennet-obtained gel.
The results for the strain of displacement of the rennet gel obtained with the use of microbial coagulant are presented in Figure 1.

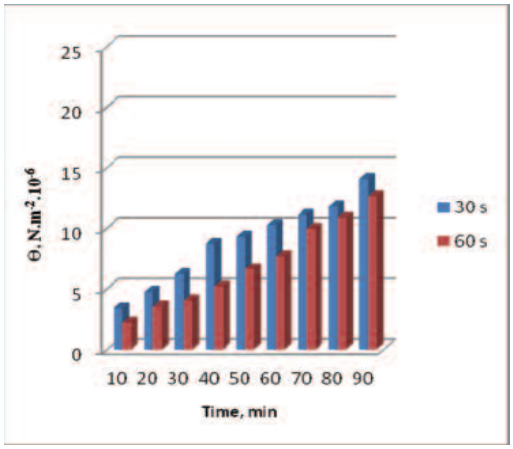


Figure 1. Strain of displacement ($Nxm^{-2}x10^{-6}$) of coagulum obtained using microbial chymosin.

The slow rate of compaction of the rennet gel with the use of the microbial enzyme resulted in low values of the strain of displacement – from 3,42 ± 0,94 $Nxm^{-2}$ x $10^{-6}$ to 14,10 ± 1,04 $Nxm^{-2}$ x $10^{-6}$ for a measuring time of 30 s and from 2,28 ± 0,81 to 12,69 ± 1,14 $Nxm^{-2}$ x $10^{-6}$ - for 60 s. The use of this type of milk-clotting enzyme led to a formation of rennet gel having a structure very easy to destroy and releasing very high quantities of lactoserum. This milk coagulant showed significant variations of the values for the strain of displacement. In comparison with the other two clotting enzymes, the differences observed and related to the examinated factor were more noticeable and essential for the gel structure.

The results for the strain of displacement of rennet gel obtained with the use of bovine coagulant are presented in Figure 2.

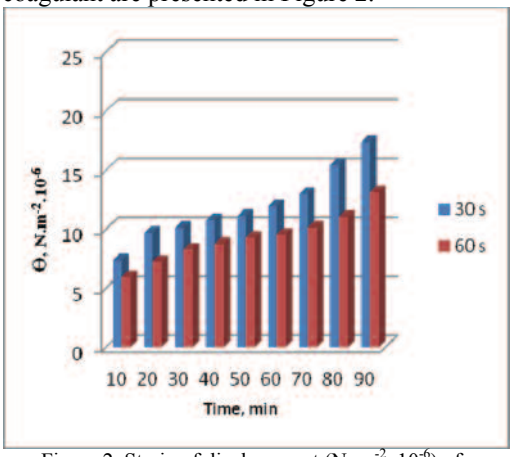


Figure 2. Strain of displacement ($Nxm^{-2}x10^{-6}$) of coagulum obtained using calf chymosin

Using the calf chymosin, it was observed that in comparison with the enzyme with microbial origin, the firming process of the gel was faster and the gel structure separated low quantities of milk serum. The strain of displacement and the variation of the obtained values were from 7,37 ± 0,60 to17,38 ± 1,55 $Nxm^{-2}$ x $10^{-6}$ for 30 s and from 5,94 ± 0,64 to 13,14 ± 1,25 $Nxm^{-2}$ x $10^{-6}$ for 60 s. The gel formed with the use of bovine chymosin showed significant differences of the values of the strain of displacement during the period of 50 min for both measured times (30 s and 60 s). The values for the calf chymosin were statistically insignificant 50 min after the initial coagulation and 70 min after it for the enzyme with microbial origin.
The results observed for the enzyme with camel origin described a process of formation of the rennet gel with intermediate characteristics - between these of a rennet gel formed with the use of bovine and with the use of microbial chymosin. The results for the strain of displacement of this enzyme are presented in Figure 3. The coagulum had a thick structure and a very low quantities of serum is separated. At the end of the experiment, the values of the strain of displacement were the highest compared to the values presented for the other two enzymes - 23,78 ± 1,84 $Nxm^{-2}$ x $10^{-6}$ for 30 s and 15,76 ± 1,64 $Nxm^{-2}$ x $10^{-6}$ for 60 s.

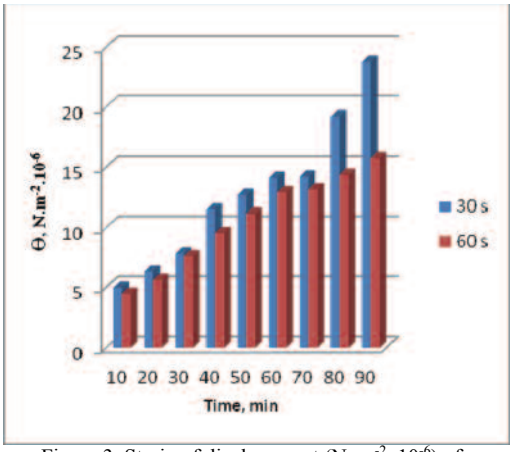

Figure 3. Strain of displacement ($Nxm^{-2}x10^{-6}$) of coagulum obtained using camel chymosin

The differences between the three studied enzymes were that the gel formed with the use of camel chymosin had no statistically significant differences between the values of the strain of displacement 20 min after the initial coagulation until the end of the experiment for both times of measurement - 30 s and 60 s.

## CONCLUSIONS

The results of the penetrometric study with the use of three different genetic variants of milk-clotting enzymes indicate that during the experiment (90 min), the rennet gels obtained with the enzyme with camel origin have the most significant and uniform rate of compaction and the coagulum releases small amounts of serum.

The serum released from the gel during the penetrometry affects measurement accuracy.

The results obtained for a period of 30 s indicate better stability of the experimental data, and shortening the time for its implementation. These results are of great importance when the enzyme of microbial origin was used, because of the amount of serum released from the gel during the times of measurement - 30 s and 60 s.

The results obtained by the conducted experiment of the rennet gel are important for modification of the method of determination of the strain of displacement; also, their application suggests new modification of the method, related to changing the time of penetration from 60 s to 30 s.

## REFERENCES

Burgess K.J., 1978. Measurement of the firmness of the milk coagulum. J Fd Sci.Technol., 2, 129-134.

Dejmek P., 1987. Dynamic rheology of rennet curd. Journal of Dairy Science, 70, 1325–1330, Netherlands.

Grandison A.S., Ford G.D., Owen A.J. & Millard D. 1984. Chemical composition and coagulating properties of renneted Friesian milk during the transition from winter rations to spring grazing. Journal of Dairy Research, 51, 69–78.

Green M.L., 1982. Assessment of two instruments for continuous measurement of the curd firming of renneted milk. J. Dairy Res., 49:127.

Hyldig G., 1993. Rennet coagulation – Effect of Technological Parameters on the Enzymatic Reaction and Gel Formation in Milk and UF Concentrates, PhD Thesis, The Royal Veterinary and Agricultural University, Copenhagen, Denmark.

Kappeler S.R., van den Brink H. (J.) M., Nielsen – Rahbek H., Farah Z., Puhan Z., Hansen E.B., Johansen E., 2006. Characterization of recombinant camel chymosin reveals superior properties for the coagulation of bovine and camel milk. Biochemical and Biophysical Research Communications, 342, 647–654.

Kowalchyk A.W., and Olson N.F., 1978. Firmness of enzymatically-formed milk gels measured by resistance to oscillatory deformation. J. Dairy Sci. 61:1375.

Law A. B., Tamime Y. A., 2010. Technology of Cheesemaking. The Production, Action and Application of Rennet and Coagulants. Vol. 2, 100-101.

Lomholt S.B. & Qvist K.B., 1997. Relationship between rheological properties and degree of kappa casein proteolysis during renneting of milk. Journal of Dairy Research, 64, 541–549.

Olson N. F., 1982. Cheese making procedures that affect yield. 5th Bienn. Cheese Ind. Conf., Utah State Univ., Logan.

Storry J.E., Grandison A.S., Millard D., Owen, A.J. & Ford G.D., 1983. Chemical composition and coagulating properties of renneted milks from different breeds and species of ruminant. Journal of Dairy Research, 50, 215–229.

Todorov T., 1975. Възможности за ускоряване и механизиране на процеса пресуване при производство на бяло саламурено сирене. Разработване и изпитване на прибор за измерване здравината на подсирка от краве мляко. Докторска дисертация, 58-62.

Valkova-Jorgova K.A., Kuzelov K., Vasilev D. Yordanov, K. Danov, 2005. Influence of a Microbial Enzyme Preparation upon solubility of myofibrillar proteins of filling mass of meat cans. Scientific Works, UFT-Plovdiv, Vol. LII, 2, 25 – 30.

van Dijk H.J.M., 1982. Syneresis of Curd, PhD Thesis, Wageningen, Agricultural University, Wageningen.

Yun S., Ohmiya K., Shimizu S., 1982. Role of b-casein in milk curdling. Agric. Biol. Chem., 46- 443.

Zoon P., van Vliet T. & Walstra P., 1988. Rheological properties of rennet-induced skim milk gels. Introduction. Netherlands Milk and Dairy Journal, 42, 249–269.