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INFLUENCE OF THE TIME FOR INITIAL COAGULATION AND RENNET GEL COMPACTNESS ON THE PROTEIN LOSSES IN WHEY

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Abstract

Analyses of the possibilities for early cutting of rennet gel in the production of cheese were made, in order to achieve more efficient use of the technological equipment. The moment of cutting the coagulum was determined by studying the rheological properties of the gel (strength, compactness, elasticity). An increase in the losses of dry matter in whey was observed in early cutting of the coagulum, result of the fragile microstructure of the milk gel.
The influence of the time for initial coagulation and rennet gel compactness on protein (casein) losses in whey was examined during enzymatic coagulation of cow's milk. The amount of used milk-clotting enzymes of calf, camel and microbial origin was 30 $cm^3.10^{-2}.dm^{-3}$. Cutting of the gel was carried out after15, 22, 30 and 60 min. The final tested value was indicated as control.
For the three milk coagulants studied, inversely proportional alteration of protein losses was established, for a time of initial coagulation between 520-630 s. A tendency to minimize the protein losses in whey was monitored by increasing the compactness and the strength of the rennet gel, which was significantly influenced by the type of used coagulants.
The obtained results can be used to analyze, predict, and modificate the technological process in cheese practice and production, particularly for production of hard-type cheeses.

Key words*: rennet gel, mechanical processing, early cutting, enzymatic coagulation, milk coagulants.*

INTRODUCTION

The enzymatic coagulation of milk and the processing of the obtained rennet gel are basic technological operations determining the yield and quality of the final product in the production of hard cheeses. One of the factors that influence the amount of the final product is the losses of dry matter in whey. They are determined primarily by loss of total protein, casein and milk fat (Mona al., 2011).
The rheological properties of the curd (density, strength, elasticity) define the initial time of the mechanical treatment of the coagulum (cutting) (Castillo al., 2004).
The determination of the rheological properties is performed by various methods - penetrometric, ultrasonic, optical, viscometric, etc. (Storry, 1982; Richardson, 1985; James, 1996). The losses of protein in whey increase if the time of cutting is inaccurately determined. The protein losses originate mainly from the process of cutting and the separation of the whey from the obtained gel (draining). The losses are determined by the hardness and strength of the gel and the nature of the milk-clotting enzyme, when the same means of mechanical treatment (cutting and stirring rate) are used (Tunick, 2000; Law, 2010).
There is an increase of losses of casein in whey, depending on the time of accelerated initial coagulation (240 s) and in relation to the cutting time studied. For samples with initial coagulation at a moderate rate and characteristics (520÷630 s) was found inversely proportional alteration in protein losses, depending on the cutting time of the coagulum (Panayotov, 2012).
The cutting time affects the losses of dry matter in whey, the moisture content of the hard cheeses, the yield and the quality of the cheeses.
The rate of cutting and stirring affects the dimensions of the particles of the coagulum, which results in an increase of the losses of protein in whey (Johnston, 2001).
Early cutting of the rennet gel increases the impact of the mechanical operations on the

rennet gel, reduces the size of the particles of the coagulum and increases the loss of protein in whey (Johnston, 2001; Castillo, 2006).
The extension of the cutting time has a positive effect on increasing the yield in the production of hard cheeses. Performing later cutting results in the formation of dense and fragile gel that separates whey difficulty, leading to a high water content, hindering the process of ripening, resulting in deterioration of the final product quality (Macedo, 1997).
The aim of the research study was to determine the protein losses in whey depending on the time for initial coagulation and the thickness of the obtained rennet gel.

MATERIALS AND METHODS

For the purpose of the experiment was used cow milk, analyzed by ultrasonic analyzer, with the following physico-chemical parameters: protein–3.3%; fat–3.7%; non-fat solids–8.60%; density–1.028 $g \cdot cm^{-3}$; titratable acidity–17°T; pH–6.76. The milk was normalized at a fat content of 3.6% and dry matter of 12.5% was achieved.
Determination of the total protein content (casein and soluble proteins) was carried out by the Kjeldahl method, using H_2SO_4 0.1 n, with a relative density of 1.84 and 33% solution of NaOH 0.1 n.
The initial coagulation was determinate using the Berridge method (Berridge, 1952).
The density of the rennet gel was defined by a penetrometer, having a cylindrical shape with $F=2 \cdot 10^{-4}$ m^2, m=0.0139 kg and k = 0.5 $N \cdot kg^{-1}$, where F-working surface, m^2; m-mass, kg; k-constant, $N \cdot kg^{-1}$.
The strain of displacement Θ was calculated by moving the operating body (h): $\Theta = k \cdot m \cdot h^{-2} = 0.00695 \cdot h^{-2}, N \cdot m^{-2}$ (Todorov, 1975).
Milk coagulants used in the experiment (in amount of 30 $cm^3 \cdot 10^{-2}$ dm^{-3}): calf chymosin, pepsin and enzymes from microbial and camel sources (market preparations, produced by Chr. Hansen) with activity 1:10000. Each milk sample had a volume of 1000 cm^3.
Cutting of rennet gel was performed on 15, 22, 30 and 60 minutes from the introduction of the milk-clotting enzymes.
Statistical Data processing was carried out by mathematical software SigmaPlot 11.0.

RESULTS AND DISCUSSIONS

An experiment was conducted to study the protein losses in whey in correlation with the time for initial coagulation and the hardness of obtained rennet gel using various types of milk-clotting enzymes with different characteristics.
The quantities of the total protein and casein losses at different values of initial coagulation time for each tested milk-clotting enzymes were defined.
In Figures 1 and 2 are presented the results for the protein and casein losses according to the time for initial coagulation using four different genetic variants of milk coagulants.
The values for the initial coagulation with the use of calf chymosin, pepsin, and enzymes from microbial and camel sources vary in the range of 520÷650 s.
With the initial coagulation time of 648 s, when pepsin was used, high levels of protein losses were observed during the experiment, also found in previous studies of Panayotov al. (Panayotov al, 2012). The lowest values for the protein losses were marked for the enzyme from camel source, with time for initial coagulation 520 s. Similar correlation between the initial coagulation time and protein losses was presented by the other two tested milk-clotting enzymes. The obtained values ranged between 1.1 ÷ 1.2%.
Differences were observed between the studied parameters, for each of the milk-clotting enzymes applied, depending on the mechanical treatment of the gel, for cutting times respectively 15, 22, 30 and 60 min.
Minimum levels of protein losses were reported at cutting the gel after 60 min from adding the milk-clotting enzymes as also was found by Law (Law, 2010). For the pepsin preparation the losses at 15 and 60 min of cutting had similar values.
Regarding the losses of casein for the four tested cutting times, the trend was similar for all milk-clotting enzymes used. Maximum casein losses were observed for cutting time of 15 min and initial coagulation time of 648 s (0,43%), corresponding to the values registered for the pepsin preparation.
Minimum casein losses were established using milk-clotting enzyme from camel

source, varying in the range of 0.15÷0.20%, because of the specificity of the enzyme, described in studies of Kappeler (Kappeler, 2006).

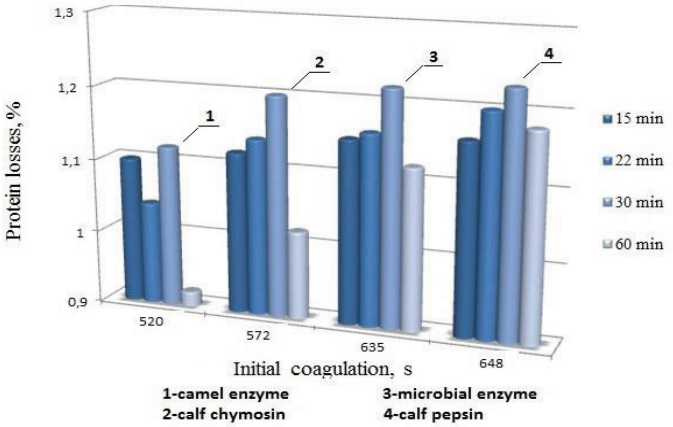

Figure 1. Total protein losses correlated with the time for initial coagulation, using enzyme of camel origin, calf chymosin, microbial enzyme and pepsin.

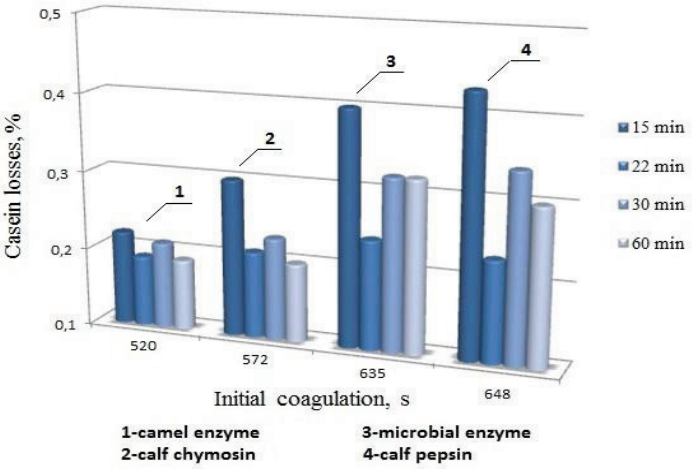

Figure 2. Casein losses correlated with the time for initial coagulation, using enzyme of camel origin, calf chymosin, microbial enzyme and pepsin

The results from the penetrometric study during the enzymatic coagulation are presented in Table 1. The values express the depth of immersion of the working body of the penetrometer using 30 $cm^3 \cdot 10^{-2} \cdot dm^{-3}$ of the milk-clotting enzymes for examination period of 60 s.

The average values of the strain displacement presented in Table 2 were calculated after mathematical processing of the data.

The results from Table 2 demonstrate that the values for the strain of displacement increase proportionally with the hardness and the strength of the obtained coagulum. Maximum values of the strain displacement (19.16 $N \cdot m^{-2} \cdot 10^{-6}$) were determinate for the camel enzyme, having a time for initial coagulation of 520 s.

The variation of the values of the strain displacement using calf chymosin and microbial enzyme was characterized by a similar rate and minimal differences in the strain of displacement during the experiment. The differences were established for cutting time 60 min after the enzymes were added in the milk, with variation of the values 18.96 ±

1.5 $N \cdot m^{-2} \cdot 10^{-6}$ for the calf chymosin and 12.07 ± 0.62 $N \cdot m^{-2} \cdot 10^{-6}$ for the microbial enzyme.
Because of the tendency of the pepsin to form labile and easily deformable rennet gel, the values for the strain of displacement at the end of the experiment using this enzyme were lower than those of the other milk coagulants tested - 9.12 ± 0.34 $N \cdot m^{-2} \cdot 10^{-6}$.

Table 1. Depth of penetration in rennet gel, using milk-clotting enzymes of microbial and camel origin, calf pepsin and chymosin

Milk-clotting enzyme type	Initial coagulation time, s	Depth of penetration (mm) in rennet gel for a cutting time (min) using milk-clotting enzymes in an amount of 30 $cm^3.10^{-2}.dm^{-3}$ for 60 s			
		15	22	30	60
Camel enzyme	520 ± 22	29,0 ± 1,8	25,0 ± 1,1	21,0 ± 1,8	18,4 ± 1,5
Calf chymosin	572 ± 23	35,2 ± 2,5	31,0 ± 2,3	27,0 ± 2,0	19,0 ± 1,8
Microbial enzyme	635 ± 22	36,5 ± 2,4	30,0 ± 2,8	27,0 ± 2,6	22,0 ± 2,1
Pepsin	648 ± 22	41, 3 ± 3,1	36,5 ± 2,4	31 ± 2,3	27 ± 2,6

Table 2. Strain of displacement in rennet gel, using milk-clotting enzymes of microbial and camel origin, calf pepsin and chymosin

Milk-clotting enzyme type	Initial coagulation time, s	Strain of displacement ($Nxm^{-2}.10^{-6}$) in rennet gel, obtained using milk-clotting enzymes in an amount of 30 $cm^3.10^{-2}.dm^{-3}$ for 60 s			
		15	22	30	60
Camel enzyme	520 ± 22	8,26 ± 0,18	9,97 ± 0,74	12, 13 ± 0,62	19,76 ± 1,64
Calf chymosin	572 ± 23	6,96 ± 0,16	8,15 ± 0,14	9,39 ± 0,42	18,96 ± 1,5
Microbial enzyme	635 ± 22	5,51 ± 0,18	8,26 ± 0,24	9,12 ± 0,34	12,07 ± 0,62
Pepsin	648 ± 22	3,88 ± 0,12	5,51 ± 0,18	8,15 ± 0,14	9,12 ± 0,34

Simultaneously with the determination of the strain of displacement were established the dependences: total protein and casein losses according to the thickness and the strength of the rennet gel.
The data obtained are presented in Figures 3-5.
The trend for the total protein and casein losses in whey, using the three milk-clotting enzymes, was similar. The highest values observed for the total protein losses was in the range 1.2 ÷ 1.4%, corresponding to a strain of displacement between 9.0÷12.13 $N \cdot m^{-2} \cdot 10^{-6}$ (or a time of cutting the rennet gel 30 min after addition of the coagulants). The losses of casein were in the range of 0.2÷0.4% (the highest values were observed at the lower values of the strain of displacement, corresponding to a cutting time 15 min after enzymes addition).
An increase of the protein losses was observed by increasing the thickness of the rennet gel, due to the formation of a gel with a friable and fragile structure. The results obtained using a calf chymosin showed differences, as the values for the total protein and casein losses were minimized at the highest values of the strain of displacement (gel with the highest hardness and strength). The process of cutting the rennet gel with higher density and strength showed a greater resistance to the cutting instruments, which leads to an increase of a total protein and casein losses in whey, resulting in a reduction in yield.
The most significant loss of protein and casein were registered using the enzyme of microbial origin, due to the rheological properties and characteristics of the formed rennet gel. The coagulum obtained in 40 min during the experiment was defined as fragile, easily deformable, with accelerated syneresis, resulting in decreasing of the values of the strain of displacement of 5.51÷12.07 $N \cdot m^{-2} \cdot 10^{-6}$.

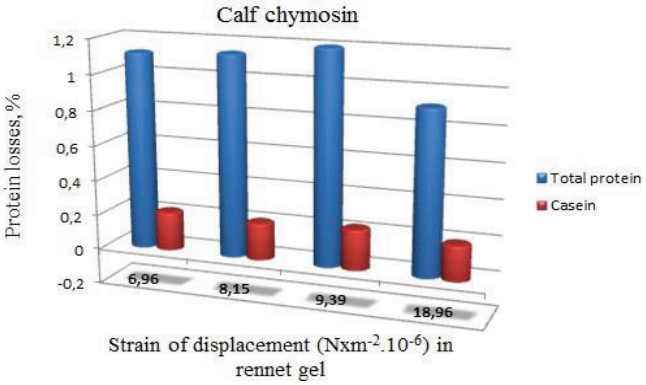

Figure 3. Total protein losses correlated with the strength of the rennet gel, using calf chymosin

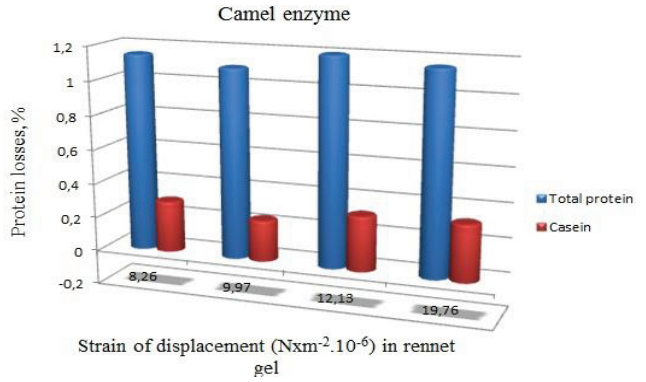

Figure 4. Total protein losses correlated with the strength of the rennet gel, using camel enzyme

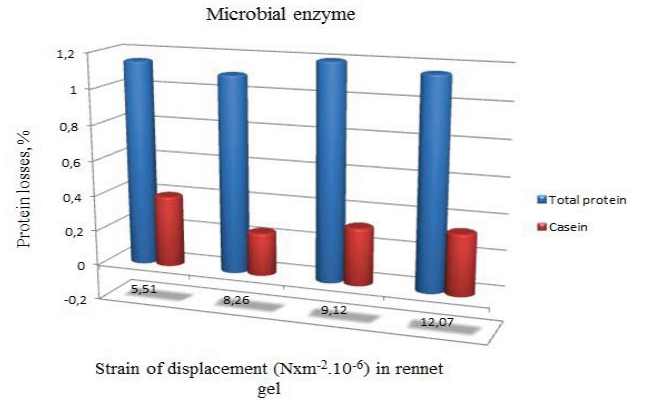

Figure 5. Total protein losses with the strength of the rennet gel, using microbial enzyme

The enzyme with camel origin leads to a formation of dense structure of the coagulum, with accelerated gelling and low syneresis. In comparison with the other tested milk-clotting enzymes, the values of the strain of displacement were maximum, which determines the minimization of the protein (1.02%) and casein (0.2%) losses.

The same pace of variation of the thickness and strength of the gels formed during the coagulation process was monitored for all the milk-clotting enzymes studied. This trend was explained by the insignificant differences in the data obtained for the total protein and casein losses in whey.

CONCLUSIONS

The conducted experiments and obtained results allow concluding that the three used milk-clotting enzymes had a similar trend in total protein and casein losses during the process of draining.

Inversely proportional variation in the values for the total protein and casein losses was monitored for the times of initial coagulation of 520÷650 s. Using enzyme with camel origin the losses were minimized, related to the acceleration of the phase of initial coagulation.

By increasing the compactness and strength of the rennet gel, the losses of protein in whey were decreased, influenced first by the milk coagulant used and second by the cutting time of the rennet gel. The late cutting of the coagulum, results in an increase of losses of dry matter in whey, due to the formation of a gel with a friable structure.

The obtained results can be used to analyze, predict and modify the technological process in cheese making practice, for the production of hard cheeses, using the three investigated enzymes.

To minimize the protein and casein losses the obtained coagulum can be cut in the earlier phase of the coagulation process, which provides minimal resistance to the cutting instruments and devices. The mentioned conditions create opportunities to maximize the protein component, increase yields and improve the quality characteristics of the final product.

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