Influence of the Amount of Rennet, Calcium Chloride Addition, Temperature, and High-Pressure Treatment on the Course of Milk Coagulation

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Abstract

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Coagulated gel was characterised using an oscillation rheometer Rheostress RS 150. The influence was monitored of the additions of rennet and $CaCl_2$, of temperature, and of high-pressure treatment on the properties of coagulated gel. Parameters of coagulation were assessed, i.e. elastic modulus G', the time of the start of coagulation t_0, and the time constant of congealation τ. In monitoring the influence of increasing additions of rennet and $CaCl_2$, and of temperature increase, it was found that the time of the start of coagulation t_0 and the time constant of congealation t decreased. The limiting shear storage modulus G'_{max} was practically constant at various amounts of $CaCl_2$ and was moderately decreasing with increasing temperature and rennet addition. This effect was possibly caused by the fact that the limiting value is a regression parameter extrapolated to infinity. The real values of the modulus for a given time increased with increasing levels of calcium chloride added. Milk treated by the pressure of 600 MPa for 10 min formed, during coagulation, a more solid gel, the shear storage modulus being, almost by 100% higher as compared with untreated milk. Value of the time constant of congealation was practically halved.

Keywords: coagulation; milk; rennet; calcium chloride; temperature; high pressure; shear storage modulus; time of the start of coagulation; time constant of congealation

In the past, several researchers tackled this matter. One example is described in the paper by ADAM *et al.* (1984) who measured the rigidity of gel by a non-destructive careful periodical pressing of the coagulate layer by 4% using the equipment Instron 1140.

In the industrial practice, calcium chloride is added to milk for an adjustment of the content of calcium ions. The milk is treated before renneting which includes the adjustment of the fat content, homogenisation of the milk fat, the adjustment of the renneting capability by the addition of $CaCl_{2}$, the microbial treatment – pasteurisation and the addition of pure cheese culture, the adjustment of the milk temperature (KNĚZ & SEDLÁČKOVÁ 1991). To milk, such dose of rennet is added as to coagulate it during a certain period of time forming curd of the required breakage and rigidity. Rennet is added to tempered milk in a diluted state under continuous stirring. During the production of sweet cheese (majority of cheeses produced nowadays), milk proteins coagulate as a result of the rennet enzymes activity under a physical-chemical joint performance of lactic acid. pH of milk should be in the range of 6.2–6.5.

As rennet, originally only the extract from dried stomachs of suckling calves was used containing predominantly the enzyme chymosin that coagulates the soluble protein casein to the insoluble para-casein. The lack of this classic rennet together with the rapidly increasing world production of cheese called for new sources of rennet enzymes; for this reason, microbial rennet substances are used nowadays. The efficiency is an important property of rennet and is called the strength of coagulation. This denotes the amount of cm^3 of fresh milk of a certain acidity that, at the temperature of 35°C, will coagulate into a solid gel after the addition of 1 cm^3 of liquid or 1 g of solid rennet during 40 min (KNĚZ & SEDLÁČKOVÁ 1991).

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The difference between rennet (sweet cheese) and acid (quark) coagulation will appear in the resulting consistency of the product – cheese has elastic consistency, quark has plastic consistency (KNĚZ & SEDLÁČKOVÁ 1991). Cheese due to its composition, especially the content of full-value proteins of animal origin and high contents of mineral substances and vitamins, belongs to the food products of the highest value. For this reason, it is important to monitor and optimise the process of cheese production and thus obtain products of a high value.

The matter of monitoring the process of milk coagulation was dealt with in the past by several works. GONCHAROV and TABACHNIKOV (1974) investigated the rigidity of gel during coagulation with rennet. They studied the course of rigidity in relation to the time of coagulation, the temperature of coagulation, and pH of renneted milk. Also, they dealt with synaeresis (separation of liquid from gel). Viscosity of coagulated milk was measured using Reotest 2. They concluded that the best structural and mechanical properties of the coagulate were reached after the time period of 2.5–3 hrs, and that the formation of structure was the fastest at pH 5.8–6.

Another work by TABACHNIKOV and DUDNIK (1975) dealt with the influence of the titration acidity on milk renneting. To 200 ml, they added an initiator, 0.2 ml of 40% $CaCl_2$, and 1.2 ml of 1% solution of chymosin (at 36°C), and they monitored when titration acidity reached 18–26°T. The course of coagulation was monitored by the viscosity measurement and also by elasticity measurement using Tromboelastograph Tromb-1. The main conclusions were that the rate of the coagulate structure formation increased with the growth of titration acidity approx. by 8%/1°T but that the final structure of the coagulate was influenced by the acidity only to a small extent, and that the mechanical properties of the coagulate were similar in the appropriate periods of the gel formation with no regard to acidity.

KOVALECKOV and BOCHAROV (1970) investigated the influence of the additions of rennet, $CaCl_2$, and NaH_2PO_4 on coagulation. Samples of milk were coagulated by the addition of rennet (0.01–0.04 ml of the preparation with the activity of 1:100 000) with added $CaCl_2$ (25–100 mg per 100 ml) at a constant amount with increasing additions of NaH_2PO_4 preparations (10–20 mg/100 ml). Rheological properties of the coagulate were measured using the equipment Rheotest, and the volume of the separated whey was determined. An increase in the concentration of rennet caused a gradual growth of viscosity and density of the coagulate and a decrease of the rate of the separation of whey. An increase in the concentration of $CaCl_2$ caused a growth of viscosity while it had a low impact on the separation of whey; the increase in the concentration of Na_2HPO_4 caused a gradual growth of viscosity and the separation of whey decreased to a half level in the presence of Na_2HPO_4 regardless of the concentration.

The management of the milk coagulation process – by measuring the heat conductivity – was studied by CINDIO *et al.* (1987). They monitored the changes in the heat conductivity during coagulation of pasteurised and fresh milk. They investigated the dynamic and mechanical properties using the method of hot wire and the dynamic spectrometer. By comparison of the results based practically on the time of coagulation, an explanation was proposed of the microstructure based on the mechanism of the colloidal gel formation. It was possible to see the difference between fresh and pasteurised milk and to distinguish various levels of coagulation.

The above mentioned works show that various amounts of rennet and $CaCl_2$ added, as well as temperature and pH of renneted milk, influence the structure, rigidity and viscosity of the gel formed. A goal of this work was to determine the course of the enzyme coagulation of milk by oscillation measurement. We investigated the course of coagulation by measuring the elastic modulus G' in relation to time. For the measured relations, the parameters of coagulation were determined: the time of the start of coagulation t_0, the time constant τ and the limiting shear storage modulus G'_{max}; also investigated was the influence of temperature, of the amount of rennet and $CaCl_2$ additions, and of the treatment of milk by pressure.

MATERIAL AND METHODS

Equipment

- Oscillation rheometer Rheostress RS 150 (Haake, Germany), using cylinder Z41 and cup Z43)
- Digitally controlled thermometer K20, DC 50 (Haake, Germany)
- Ultra-cryostat MK 70 (Medingen, Germany)
- Digital thermometer Therm Pt 100 (Germany)
- High pressure press CYX 6/0103 (ŽĎAS, a.s., Czech Republic)

Raw materials

- Semi-fat milk 1.5% fat, homogenised, purchased in a retail outlet
- 1% solution of rennet 1:15 000 (classic rennet)
- 0.2M solution of calcium chloride $CaCl_2$

Coagulation of milk – various parameters of influence

Influence of the frequency of oscillation used (preliminary measurement). Milk was treated by the addition of 0.2M solution of $CaCl_2$ to all samples. 1900 ml of milk was well stirred with 100 ml 0.2M solution of $CaCl_2$ (basic dose is 950 ml of milk + 50 ml of $CaCl_2$). Also, 1% solution of rennet was prepared. In to a beaker, 250 ml of treated milk was always measured with its temperature adjusted to 31°C. Then a 12.5 ml of rennet solution (basic dose is 1000 ml of milk + 50 ml of rennet solution) was added, the solution was well stirred and the oscillation measurement was begun. The data found using the rheometer Rheostress were transferred into the computer using the software program Rheowin Job. The measurement was performed three times and the average value was calculated. Between the individual oscillation measurements the time step of 1 min was selected.

During this preliminary series, the influence of frequency was investigated. The values of 0.1; 0.2; 0.5; 1 Hz were chosen. The measurements were performed at a constant amplitude and, at the end of each measurement, so called sweep test was performed – i.e. the measurement at a constant tangential stress.

Influence of rennet dose. Milk for all samples was adjusted by adding 0.2M of $CaCl_2$ solution (1900 ml of milk + 100 ml of $CaCl_2$ solution). In this series, the influence was monitored of the amount of rennet added. Six samples were prepared with the additions of 20, 30, 40, 50, 60, 70 ml of rennet per 1000 ml of the adjusted milk – we measured 250 ml of the adjusted milk + the aliquot amount (i.e. 5; 7.5; 10; 12.5; 15; 17.5 ml) of 1% rennet solution. The measured rennet solution was poured in to the adjusted and tempered sample of milk. The frequency of oscillations was set to 1 Hz, the relative deformation to 3.2%, the temperature of coagulation to 31°C. The measurement was performed three times and the average value was calculated. Between the individual measurements, the time step of 1 min was selected.

Influence of $CaCl_2$ dose. The procedure for the preparation was slightly different because each sample of milk was now adjusted separately. Milk was adjusted using the following additions of 0.2M solution of $CaCl_2$: 20 ml of $CaCl_2$ + 980 ml of milk, 30 ml + 970 ml, 40 ml + 960 ml, 50 ml + 950 ml, 60 ml + 940 ml, 70 ml + 930 ml). 250ml samples of adjusted were prepared (i.e. 5, 7.5; 10; 12.5; 15; 17.5 ml of $CaCl_2$ which were made up with milk to the total volume of 250 ml). The samples were warmed to and kept at the temperature of 31°C. To each, conditioned sample of 250 ml volume in the beaker, 12.5 ml of 1% rennet solution was added (basic dose of 50 ml per 1000 ml of adjusted milk). The frequency of oscillation was set to 1 Hz, the relative deformation to 3.2%. The masurement was performed three times and the average value was calculated. Between the individual measurements, the time step of 1 min was selected.

Influence of temperature. Samples were prepared in a similar way as in the preliminary measurement, i.e. all milk samples were adjusted in the ratio of 50 ml of 0.2M solution of $CaCl_2$ per 950 ml of milk. Into each conditioned sample of 250 ml volume the same amount was added of 1% rennet solution – 12.5 ml per 250 ml of the adjusted milk (basic dose is 50 ml of rennet per 1000 ml of adjusted milk). The temperature of coagulation for the individual samples was selected as follows: 27.5; 30; 32.5; 35; 37.5 and 40°C. The measurement was performed three times and the average value was calculated. Between the individual measurements, the time step of 1 min was selected.

Influence of very high pressure. In this series, the goal was to find whether the treatment of milk with a very high pressure has an influence on its coagulation. Two samples were prepared of milk treated with the pressure of 600 MPa for 10 min and, as the control samples, 2 samples not treated by pressure. The high-pressure treatment was performed at room temperature. The initial temperature of milk was about 5°C and the compression heating could lead to an increase of temperature up to 20°C. The pressure decay resulted in a decrease of the milk temperature back to about 5°C.

All four samples were further treated in the same way, i.e. to 475 ml of milk, was added 25 ml of 0.2M of $CaCl_2$ solution. Then, this 250 ml of the conditioned milk was added into 12.5 ml of 1% rennet solution. The samples were kept at the temperature of 31°C. The frequency of oscillation was set to 1 Hz, the relative deformation to 3.2%. The measurement was performed three times and the average value was calculated. Between the individual measurements, the time step of 1 min was selected.

RESULTS AND DISCUSSION

The time course of the shear storage modulus measured was replaced in the increasing part by the exponential function of the following form:

$$G' = G'_{max}\left(1 - e^{-\frac{t - t_0}{\tau}}\right) \qquad (1)$$

where: G'_{max} – limit shear storage modulus
t_0 – time of the start of coagulation (called also the lag phase)
τ – time constant

For the determination of these factors, non-linear regression was used performed by the application of the software program Datafit 6.1 (Oakdale Engineering, USA). This software program also determines the confidence intervals of the parameters. The numerical values of the parameters together with the confidence intervals are given in Tables 1–3. It is apparent from these tables that the confidence intervals are smaller than about 5% of the determined values of the parameters given. It confirms that model (1) describes correctly the coagulation process. The comparison of the regression models with the data is apparent from Fig. 1 in which the lines represent the regression model for the data given. It can be seen that model (1) is not capable to describe the initial parts of the coagulation process, mainly due to the slow coagulation. This is caused by the fact that in the real situation, coagulation is not exactly of the first-order process. Nevertheless, mod-

Table 1. Parameters of regression dependence of modulus G' on time as a function of frequency of oscillation during coagulation of renneted milk (50 ml of rennet per litre of milk with $CaCl_2$) – content of fat 1.5%; temperature 31°C; pH = 6.4; relative deformation 3.2%

Frequency of oscillations (Hz)	t_0 (s)	τ (s)	G'_{max} (Pa)
0.1	451	1388	122
0.2	467	1470	127
1.0	483	1373	156

el (1) is a very good tool for the description of coagulation at later stages, and namely for the prediction of the shear storage modulus and the quality of curd at the time given (e.g. for practical purposes of the cutting time prediction).

Influence of frequency. The course of the shear storage modulus G' with time has almost the same values for frequencies 0.1 and 0.2 Hz. In Table 1, parameters are listed of the regression dependence in accordance with relation (1) as functions of frequency. From the value of G'_{max}, it is possible to see that, at the frequency of 1 Hz, the least rupture of gel occurs (the value G'_{max} is at the highest level). For the following measurements, consequently, the frequency of oscillation at 1 Hz was selected. This is probably caused by the fact that, at low values of frequency, gel is under movement for a longer time.

The shear storage modulus decreases moderately with the amplitude growing above 4%. For this reason, in all following measurements the deformation amplitude at

Table 2. Parameters of regression dependence of modulus G' on time as a function of addition of 1% rennet solution, of dose of 0.2M $CaCl_2$ solution, and temperature during coagulation of renneted milk (50 ml of rennet per litre of milk with $CaCl_2$) of the content of fat 1.5%; temperature 31°C; pH = 6.4; relative deformation 3.2%, frequency of oscillations 1 Hz

	t_0 (s) conf. interval 95%	τ (s) conf. interval 95%	G'_{max} (Pa) conf. interval 95%
Content of rennet solution (ml/l of milk with $CaCl_2$)			
20	1131 ± 6.2	2698 ± 44.0	171 ± 1.5
30	839 ± 6.0	2219 ± 30.8	165 ± 1.0
40	613 (*370) ± 5.4	1884 (*1441) ± 21.9	164 ± 0.8
50	517 (*296) ± 5.3	1875 (*1153) ± 23.1	153 ± 0.8
60	442 (*247) ± 5.8	1665 (*961) ± 23.2	152 ± 0.9
70	416 ± 6.8	1613 ± 30.4	144 ± 1.2
Dose of $CaCl_2$ solution (vol. %)			
2	1213 ± 13.2	4983 ± 235.4	157 ± 5.1
3	850 ± 7.8	2779 ± 46.4	151 ± 1.3
4	623 (*468) ± 5.4	2075 (*2138) ± 23.1	154 ± 0.7
5	504 (*389) ± 7.0	1722 (*1692) ± 28.0	152 ± 1.0
6	399 (*310) ± 5.6	1481 (*1246) ± 21.8	162 ± 0.9
7	339 ± 10.8	1327 ± 31.5	164 ± 1.3
Temperature** (°C)			
27.5	519 ± 5.6	2557 ± 24.2	152 ± 0.6
30	462 (*442) ± 10.8	1947 (*3051) ± 41.4	144 ± 1.1
32.5	417 ± 14.9	1431 ± 37.9	138 ± 1.0
35	324 (*338) ± 33.6	1177 (*727) ± 53.2	139 ± 1.1
37.5	311 ± 33.6	1022 ± 53.3	132 ± 1.3
40	300 (*234) ± 19.5	889 (*173) ± 38.6	120 ± 1.1

* in the brackets is included data that was obtained by ADAM *et al.* (1984)

** during regression, there is considered data for coagulation time up to 3000 s

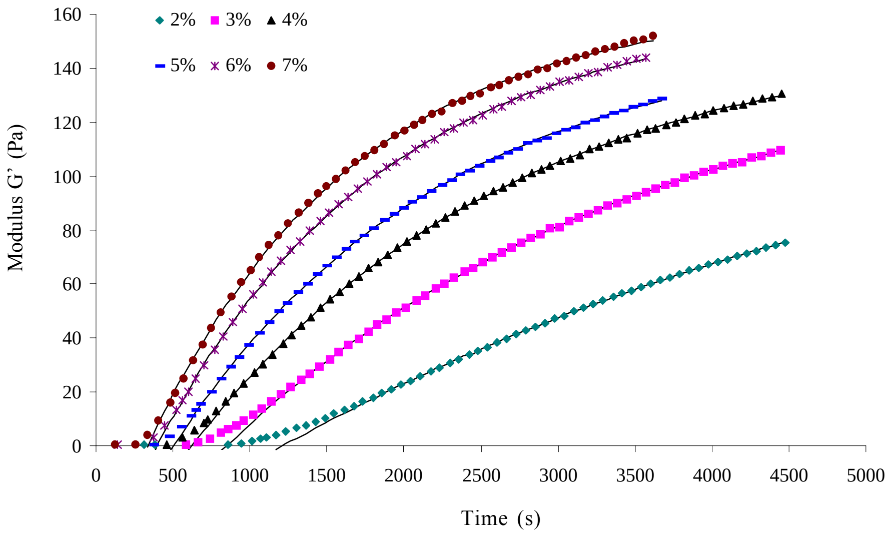

Fig. 1. Dependence of modulus G' on time and on the content of $CaCl_2$ solution (vol. %) during coagulation of milk; content of fat 1.5%; temperature 31°C; pH = 6.4; relative deformation 3.2%; oscillation frequency 1 Hz

3.2% was selected for which the shear storage modulus is not influenced.

Influence of rennet dose. Numerical values of the calculated parameters are included in Table 2. It is obvious that by increasing the dose of rennet, the time of the start of coagulation t_0 is getting shorter. Maximum decrease occurred in the time of the start of coagulation t_0 at the dose of 70 ml/l, i.e. by 63% in relation to time t_0 for the dose of rennet solution 20 ml per litre of milk, while and the decrease of the time constant of congealation was 40%. For the limiting shear storage modulus, only a moderate decrease by 16% occurred.

If we compare the time of the start of coagulation t_0 as well as the time constant of congealation τ with the results of ADAM *et al.* (1984), it is obvious that the values measured by us are always higher (Table 2). This can be caused by the use of milk with different contents of fat (1.5% instead of 1.9%).

Influence of $CaCl_2$ dose. The results of this series of samples are indicated for illustration in Figs 1–4 (the other series of samples were also processed in the graphical way, however, they are not included in this paper), and in Table 2. In Figs 1 and 2, it can be seen that, with an increasing addition of $CaCl_2$, the time of the start of coagulation is getting shorter. The time of the start of coagulation t_0 was the shortest for the $CaCl_2$ dose of 7% volume, i.e. by 72% in relation to time t_0 for the dose of calcium chloride of 2% volume. Also decreasing in the time constant of congealation τ (Fig. 3) – by 73%. From Fig. 4, it is obvious that the limit shear storage modulus G'_{max} does not practically change within the measured range. From the results mentioned above it follows that the process of coagulation starts and ends in a faster way with the increasing additions of calcium chloride, while the final limit value of the gel rigidity is not influenced practically.

If we compare the time of the start of coagulation t_0 and the time constant of congealation τ with the results of ADAM *et al.* (1984), it can be seen in Table 2 and Fig. 2 that the values of t_0 measured by us are always higher. On the contrary, the values of the constant of congealation τ measured by us, are almost the same (Table 2 and

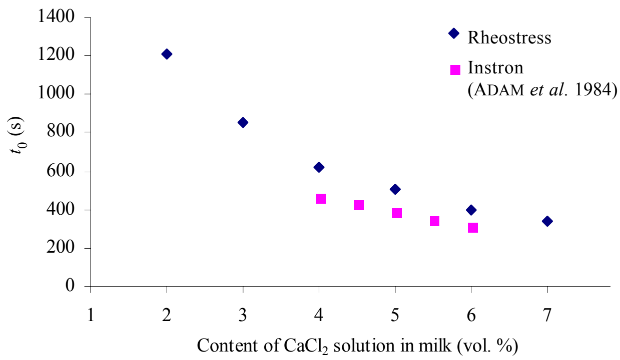

Fig. 2. Dependence of t_0 on the content of $CaCl_2$ solution in milk during coagulation

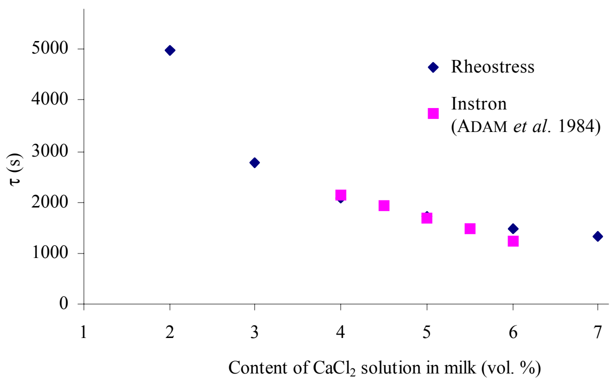

Fig. 3. Dependence of τ on the content of $CaCl_2$ solution in milk during coagulation

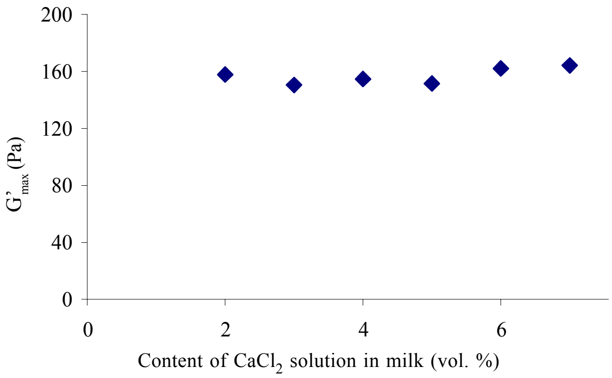

Fig. 4. Dependence of G'_{max} on the content of $CaCl_2$ solution in milk during coagulation

Fig. 3) in comparison with the literature data of ADAM *et al.* (1984).

Influence of temperature. The influence of temperature on the course of coagulation is considered one of the most important factors. It varies depending on the kind of the cheese produced (28–32°C soft cheese, 30–34°C hard cheese) – KNĚZ and SEDLÁČKOVÁ (1991). With increasing temperature, the time of coagulation is first getting shorter but approximately from the temperature of 50°C, the time of coagulation is, on the contrary, getting, longer in the dependence on the kind of rennet. From this reason, we limited our measurements to the practically used range of temperatures from 27.5 to 40°C in which this influence has a positive impact on the increase of the rennet performance.

In Table 2, the calculated parameters of coagulation are clearly organised. The time of the start of coagulation t_0 was the shortest at 40°C, i.e. by 42% in relation to time t_0 for the temperature of 27.5°C. The time constant τ decreased by 63% and the limiting shear storage modulus decreased by 21%.

If we compare the time of the start of coagulation t_0 and the time constant of congealation τ with the results determined by ADAM *et al.* (1984), it is obvious that the values of t_0 measured by us practically correspond to their results. A different situation appears in the case of the parameter τ. The comparison shows that the values are quite different (Table 2).

Milk treated by very high pressure. In Table 3, the parameters of coagulation are listed in Fig. 5, the dependence of

Table 3. Parameters of regression dependence of modulus G' on time as a function of way of treatment during coagulation of renneted milk (50 ml of rennet per litre of milk with $CaCl_2$) of the content of fat 1.5%; temperature 31°C; pH = 6.7; relative deformation 3.2%

Way of treatment	t_0 (s) conf. interval 95%	τ (s) conf. interval 95%	G'_{max} (Pa) conf. interval 95%
Not treated by pressure	410 ± 18.4	1640 ± 41.4	167 ± 1.1
	412 ± 18.4	1746 ± 44.3	170 ± 1.2
Treated by pressure 600 MPa/10 min	392 ± 4.1	701 ± 7.0	300 ± 0.4
	445 ± 5.0	843 ± 8.1	305 ± 0.5

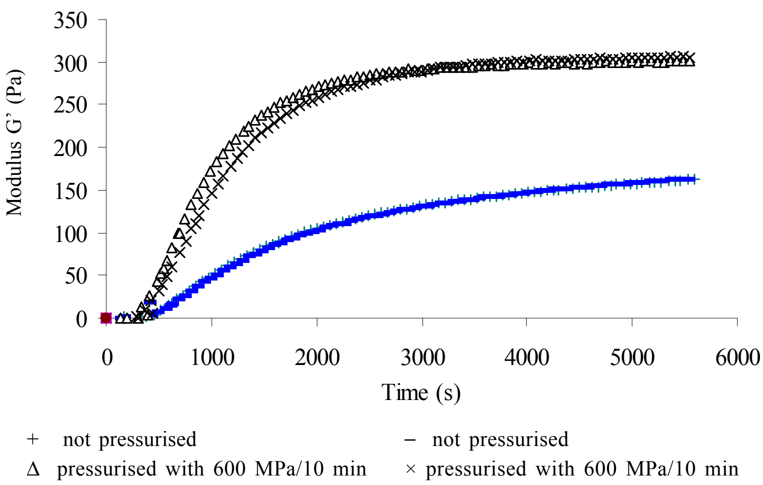

Fig. 5. Dependence of modullus G' on time and various modes of treatment during coagulation of renneted milk (50 ml of rennet per litre of milk) of the content of fat 1.5%, temperature 31°C, pH = 6.7; relative deformation 3.2%

the shear storage modulus G' on time. It is obvious that the milk treated with a very high pressure has the modulus *G'* by almost 100% higher. The time constant τ is higher by more than 100%, for the milk not treated by pressure.

Conclusions

The parameters of coagulation t_0, τ and G'_{max} make it possible to roughly assess the moment of the start of coagulation and almost exactly predict the rigidity of curd in the course of the further process. For practical reasons, it is good to compare the rigidities reached by curd under given conditions for the selected period of time. This method could be used for optimising the technological conditions with regard to achieving the required rigidity of curd. The three regression parameters of model (1) represent very well the whole coagulation curve with the exception of the very beginning of the process. These parameters can serve for the data condensation and the reproduction of the whole coagulation curve (calculation of modulus at selected time).

When monitoring the influence of the dose of rennet, the amount of $CaCl_2$, and the increase of temperature, it was found that the time of the start of coagulation t_0 and the time constant of rigidity are falling down. The limiting shear storage modulus G'_{max} at various amounts of dose of $CaCl_2$ was practically constant and, when monitoring the influence of temperature and the addition of rennet, it is falling down moderately. This effect is caused by the fact that this parameter represents the extrapolated value of the modulus to infinity while the data were measured for the real period of time. If we take into account the modulus at a given time, e.g. for 40 min, it is, found to increase with the increasing dose of $CaCl_2$.

The influence was also investigated of the milk treatment with a very high pressure. Milk treated with the pressure of 600 MPa for 10 min formed a more solid gel during coagulation. The shear storage modulus was, in comparison with untreated milk, by almost 100% higher. The time constant of congealation was practically at a half level. This effect could be used to spare a dose of rennet in the process of the pressure pasteurisation of milk.

List of symbols

t_0 – time of coagulation start (s)
G' – shear storage modulus (Pa)
G'_{max} – limiting shear storage modulus (Pa)
τ – time constant of congealation (s)

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Souhrn

LANDFELD A., NOVOTNÁ P., HOUŠKA M. (2002): **Vliv dávky syřidla, přídavku chloridu vápenatého, teploty a ošetření vysokým tlakem na průběh koagulace mléka**. Czech J. Food Sci., **20**: 237–244.

Vlastnosti koagulovaného mléčného gelu byly měřeny oscilačním reometrem Rheostress RS 150. Byl sledován vliv přídavku syřidla, chloridu vápenatého, teploty a zpracování mléka vysokým tlakem na vlastnosti vznikajícího gelu. Byly definovány parametry koagulace: elastický modul pružnosti ve smyku G', doba počátku koagulace t_0 a časová konstanta koagulace τ. Čím vyšší je přídavek syřidla, chloridu vápenatého a teplota, tím je kratší doba počátku koagulace a časová konstanta koagulace. Limitní hodnota modulu pružnosti G'_{max} nebyla prakticky ovlivněna přídavkem chloridu vápenatého a mírně klesala s rostoucí teplotou a přídavkem syřidla, což ale může být způsobeno pouze tím, že tento parametr představuje regresní extrapolaci do času nekonečno. Hodnoty modulu pružnosti pro určitý časový okamžik s přídavkem chloridu vápenatého vzrůstaly. Mléko předem ošetřené tlakem kolem 600 MPa po dobu 10 min vytvořilo po přidání syřidla mnohem pevnější gel než mléko tlakem neošetřené. Modul pružnosti byl dokonce o 100 % vyšší a časové konstanty prakticky poloviční.

Klíčová slova: koagulace; mléko; syřidlo; chlorid vápenatý; teplota; vysoký tlak; elastický modul pružnosti; doba počátku koagulace; časová konstanta vytužování

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