Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 11, 2017, no. 1, p. 368-372
doi: https://dx.doi.org/10.5219/742
Received: 10 February 2017. Accepted: 13 April 2017.
Available online: 29 June 2017 at www.potravinarstvo.com

ISSN 1337-0960 (online)

QUALITY PARAMETERS OF CURD

Roman Pytel, Šárka Nedomová, Vojtěch Kumbár, Libor Kilián

ABSTRACT

The aim of this work was measurement of the curd firmness prepared by different volume of rennet solution and comparison of differences of curd firmness between these volumes. Further, it was observed the influence of different volumes of rennet up to thevolume of whey release, curd firmness, dry matter of curd and dry matter of whey. The composition of milk was determined according ISO and Czech state standard. Dry matter content (%) was determined by gravimetry, in drying oven at 102 °C to constant weight (ISO 6731:2010), the protein content (%) by Kjeldahl´s method (EN ISO 8968-1:2002), content of fat (%) by Gerber´s acidobutyrometric method (ISO 2446:2008), content of lactose by polarimetry, titratable acidity by titration Soxhlet-Henkel method, pH and calcium content in milk ($g.L^{-1}$) was determined by complexometric titration with flueroxone as an indicator according to Czech state standart No 57 0530. For coagulation was used microbial rennet CHY-MAX^{R}M 200. There were measured: curd firmness, rennet coagulation time, curd quality, volume of released whey, weight of curd, dry matter of curd and dry matter of whey. Different volumes of rennet solution had influence on curd firmness. Curd firmness (volume of rennet solution) had no significant influence on curd quality (five grade scale). Curd firmness had influence on volume of whey release (mL) out of the curd. With the increasing curd firmness ~~s~~ increased the volume of whey released from the curd. The volume of rennet solution had an influence on weight of curd. Curd dry matter raised with higher volume of rennet solution. Volume of added rennet solution had no statistically significant effect on the change of whey dry matter.

Keywords: milk; curd firmness; volume of rennet; microbial rennet

INTRODUCTION

Milk coagulation properties are one of the most important technological properties, which include rennet coagulation time, time to curd firmness 20 mm and curd firmness 30 min after enzyme addition **(Bittante, 2011)**.

Theessential characteristic step in manufacture of all cheese varieties is cogulation of the casein component of the milk protein system to form a gel which entraps the fat, if present. Coagulation may be achieved by acidification to pH 4.6, acidification to about pH 5.2 incombination with heating to 90 °C or limited proteolysis by selected proteinases **(Fox et al., 2004)**.

Gels are semisolid gelled structures with some shape retention and elasticity. In the lyogel the casein particles are oriented in three-dimensional honeycombed gel structure whose interstices are filled with water.

The structure of the gel is created during milk coagulation has a significant influence on all later operations such as whey discharge, the ripening process and hole formation **(Spreer, 1998)**. The gel formation is accompanied by a number of physicochemical changes, including hydrolysis of κ-casein, increase in the concentration of the glycomacropeptide; aggregation of the sensitized para-casein micelles, increases in viscosity and elasticity of the milk, and a decrease in the ratio of the viscous to elastic charakter of the milk **(Fox et al., 2000)**.

Gel can be characteristic by various parameters. Important is structure, especially the coarseness of the network, which can be given as the pore size (disturbution). The rheological properties are also essential. One mostly determines the elastic shear modulus or stiffness. This parameter is measured at very small strain. At larger strain, the proportionality between stress and strain is lost, and when the structure stress or strength of the material is reached, the test piece breaks; the fracture strain is also a relevant variable **(Walstra et al., 2006)**.

Curd firmness is dependent on the coagulation temperature and milk quality, it is equally dependent on the amount and quality of rennet. Curd firmness rises to a certain limit from the moment of precipitation proportionally with time. The curd firmness is higher with shorter time of coagulation **(Teplý et al., 1976)**. Good gel-forming properties are characterized by a relatively rapid coagulation time, high-curd firmning rate and a high-curd firmness or strength after a given renneting time **(Fox et al., 2000)**. The gel structure is affected by treatments of milk before renneting, as heating regime or the the homogenization. These treatments influence milk and change their gelation behaviour **(Donato and Guyomarc'h, 2009)**.

Milk coagulation properties are strongly affected by coagulation temperature, pH, $CaCl_2$ and concentration enzyme **(Nájera et al., 2003)**. Clotting temperature between 30 – 35 °C gave gels with higher firmness (**Lucey, 2002)**. Influence to formation of curd gel consistency, have content of calcium, especially the ionic form **(Roginsky et al., 2003)**.

When is used microbial rennet for renneting, it is needed a longer time for coagulation compared with animal rennet. The curd produced using microbial rennet is softer. As well as, the structure of the curd prepared by various kinds of rennet is different **(Teplý et al., 1976)**.

Optimal curds firmness is one of the main demands in cheesemaking. Soft curd in the cheesemaking is sticky, whey is poorly dripping. In the whey is leaving more milk components. On the other hand, solid curds had an influence to quality of the finally products **(Teplý et al., 1976)**.

Fox et al. (2000) report, that for measure viscosity and curd firmness of the coagulum may be used various type of penetrometers and viscometers.

For the measured of firmness were used penetrometers, which are based on the principle of probe penetration through the test material **(Burgess, 1978; Storry and Ford, 1982)**. The force needed to achieve a given depth or total depth of penetration. The more resistant material is, the greater power need for penetration or the smaller the penetration depth. Distinguish penetrometer with a constant load or constant speed **(Krkošková, 1986)**.

The next method for measurement is determination curd firmness by tromboelastograph where is character of the curve typical for each type of rennet **(Teplý et al., 1976)**.

López et al. (1999) used in their work rotational viscometers, but this type of viscometers provide only limited information about curd firming.

For determination rennet coagulation time and curd firming is used torsion viscometers (thromboelastography or lactodynamography). The most popular torsion viscometer is Formagraph. The Formagraph is an instrument which is able to monitoring of coagulation properties of milk. This test is based on the movement of small, stainless steel, loop pendulum immersed in linearly oscillating samples of coagulating milk. The forces, which are apply to the pendulums as a consequence of formation of a gel in the moving milk sample (**McMahon and Brown, 1982)**.

The next method for predict optimal cutting time as measured by Formagraph is diffuse reflectance technique. The inflection point of sigmoidal phase of the diffuse reflectance curve was well correlated with the Formagraph measure of the rennet clotting time **(Payne et al., 1993).**

MATERIAL AND METHODOLOGY

This research was carried out in Biotechnology Pavilion M, financed by the OP VaVpI CZ.1.05/4.1.00/04.0135 project at the Department of Food Technology at Mendel University.

For this work was used raw milk of Holsteindairy cows from South Moravian region. Before analysis, milk was heated up to 40 °C and then cooled down to 20 °C for better dispersion of the fat globules. The milk used for determination of rennet coagulation time and curd firmness was analysed for some basic laboratory parameters. It was determined: dry matter content (%) by gravimetry, by drying oven at 102 °C to constant weight **(ISO 6731:2010)**, protein content (%) by Kjeldahl´s method **(EN ISO 8968-1:2002)**, content of fat (%) by Gerber´s acidobutyrometric method **(ISO 2446:2008)**, content of lactose by polarimetry, titratable acidity by titration Soxhlet-Henkel method, pH and calcium content in milk ($g.L^{-1}$) was determined by complexometric titration with flueroxone as an indicator according to **Czech state standart No 57 0530 (1974)**.

In this work was used the proteolytic enzyme, which causes precipitation of milk protein. There was used microbial rennet CHY-MAX^{R}M 200 (CHR. HANSEN, Denmark; 197 $IMCU.mL^{-1}$; BB: 5/2017). Rennet solutions were prepared daily by diluting 15 mL of rennet CHY-MAX^{R}M 200 with 85 mL deionized water.

The 100 mL of milk was equilibrated at 35 °C, after equilibrated, it was added into samples of milk of 1 mL, 2 mL or 5 mL rennet solution. The experiment was repeated five times for every volume of added rennet. In each category were prepared five milk curd samples for measurement.

There was measured the time required for the first visible flakes (visual method for determination rennet coagulation time). The renneting milk was placed into a thermostat at 35 °C. After one hour in thermostat, samples of milk were evaluated according five grade scale of rennet curd quality by **Kuchtík et al. (2008)**. Another measurement was the volume of whey, which was released from curd by syneresis. The curd was placed on a plate and there was measured firmness of curd by hand penetrometry FG-5N (SHITO, China). For penetrationwas used conical probe, which was permeated 1 cm into depth from surface. Each curd was measured five times. Here was measured the maximum value of hardness. The rate of penetration was constant. After penetration ~~it~~ was determined dry matter of curd and dry matter of whey by drying to constant weight.

The results were statistically processed by program MS EXCEL and STATISTICA version 12, by ANOVA test, especially Tukey´s test (p <0.05).

The aim of this work was measurement of the curd firmness, which was prepared by different volumes of rennet solution and comparing differences of curd firmness between these volumes. Further, there was observed the influence of different volumes of rennet to a volume of whey release, curd firmness, dry matter of curd and dry matter of whey.

RESULTS AND DISCUSSION

The milk used for this work had the following composition: dry matter 13.153 ±0.166%, content of protein was in range from 3.45 to 3.81% by Kjeldahl´s method. **Pretto et al. (2011)** showed that the average content of protein is 3.50%. The average content of fat in milk was 3.85 ±0.29% by Gerber´s acidobutyrometric method, which is slightly lower than content of fat (3.93%) by **Pretto et al. (2011)**. Lactose content by polarimetry was in range from 4.65 to 4.81%. Calcium content determined by complexometric titration in milk was in range from 1.15 to 1.33 $g.L^{-1}$. Titratable acidity by titration

Table 1 Influence of volume rennet solution to curd firmness (mN).

Volume of rennet solution	Average	Standard deviation	Minimum	Maximum	Coefficient of variation (%)
1 mL	5.91[a]	0.89	4.00	7.62	15.00
2 mL	7.51[b]	1.73	5.04	13.78	22.99
5 mL	7.24[b]	1.57	5.32	11.28	21.70

Note: [a, b] – different superscripts in a column indicate a statistically significant difference at p <0.05.

Table 2 Influence of curd firmness (volume of rennet solution) to rennet coagulation time (s).

Curd firmness (Volume of rennet solution)	Average	Standard deviation	Minimum	Maximum	Coefficient of variation (%)
5.91mN (1 mL)	128.8[a]	3.6	124.0	136.0	2.8
7.51 mN (2 mL)	71.5[b]	3.4	57.0	75.0	4.7
7.24 mN (5 mL)	36.2[c]	1.0	35.0	39.0	2.7

Note: [a, b, c] – different superscripts in a column indicate a statistically significant difference at p <0.05.

Table 3 Influence of curd firmness (volume of rennet solution) to curd quality (five grade scale).

Curd firmness (Volume of rennet solution)	Average	Standard deviation	Minimum	Maximum	Coefficient of variation (%)
5.91mN (1 mL)	1.0[a]	0.2	1.0	2.0	18.9
7.51 mN (2 mL)	1.0[a]	0.0	1.0	1.0	0.0
7.24 mN (5 mL)	1.1[a]	0.3	1.0	2.0	25.2

Note: [a] – different superscripts in a column indicate a statistically significant difference at p <0.05.

Table 4 Influence of curd firmness (volume of rennet solution) on release whey (mL).

Curd firmness (Volume of rennet solution)	Average	Standard deviation	Minimum	Maximum	Coefficient of variation (%)
5.91mN (1 mL)	20.8[a]	2.9	17.0	26.0	14.2
7.51 mN (2 mL)	24.5[b]	3.0	19.0	32.0	12.3
7.24 mN (5 mL)	27.5[c]	3.7	22.0	38.0	13.3

Note: [a, b, c] – different superscripts in a column indicate a statistically significant difference at p <0.05.

Soxhlet-Henkel method was 6.66 ±0.22 (°SH) and pH was 6.65 ±0.04.

Different volume of rennet solution had an influence on curd firmness (Table 1). 1 mL rennet solution gave a curd with firmness 5.91 mN. 2 mL of rennet solution gave a curd with firmness 7.51 mN, this value is higher than 5.91 mN. Addition of 5 mL rennet solution gave a curd with firmness 7.24 mN, which is slightly lower than the addition of 2 mL of rennet solution. Difference of curd firmness was statistically significant between 1 mL and 2 mL, 1 mL and 5 mL of rennet solution, but difference between 2 mL and 5 mL rennet solution was not statistically significant. **Teplý et al. (1976)** indicate that curd firmness is directly dependent on the concentration of the rennet. Equally, coagulation time was significantly affected by rennet concentration **(Sbodio et al., 2006)**.

In the Table 2 are shown average results of rennet coagulation time for different curd firmnesses (volume of rennet solution). Rennet coagulation times for milk used in this work are 128.8 s for 1 mL of rennet solution, 71.5 s for 2 mL and 36.2 s for 5 mL of rennet solution. It follows, that with increasing volume of rennet solution, the rennet coagulation time is shorter. According **Bujko et al. (2011)**, rennet coagulation time is evaluated as "good", because coagulation time is in range 110 – 140 s.

Curd firmness (volume of rennet solution) didnot have any significant influence to curd quality, shown in Table 3, evaluated by **Kuchtík et al. (2008)**. All curd was classified as very good and hard, keeping its shape after its removal from the container; whey was clear with yellow-greenish colour (1) or as good curd but a little softer, not keeping shape quite perfectly; whey is greenish (2). The curd quality is also affected by rennet, which is used for renneting **(Pytel et al., 2016)** and stage of lactacion, which had significant effect on all millk properties and curd quality **(Kuchtík et al., 2008)**.

Curd firmness had an influence on a volume of whey release (mL) from the curd (Table 4). With the increasing curd firmness increased the volume of released whey from the curd. The difference between groups was statistically significant (p <0.05).

The curd was weighed after 1 min drippingand then was monitored the influence of curd firmness to curd weight (Table 5). Average weight of curd prepared by 1 mL of rennet solution had 70.30 g, while the average weight of curd prepared by 2 mL of rennet solution had 68.15 g. Betweenthese group, there was no statistically significant difference. The statistically significant difference was between the weight of curd prepared by 1 mL and 5 mL of rennet solution, which had an average weight 65.52 g. There is statistically significant difference between 2 mL and 5 mL of rennet solution.

In Table 6 are shown results where was monitored the influence curd firmness on curd dry matter. Curd dry

Table 6 Influence of curd firmness (volume of rennet solution) on curd dry matter (%).

Curd firmness (Volume of rennet solution)	Average	Standard deviation	Minimum	Maximum	Coefficient of variation (%)
5.91mN (1 mL)	15.645[a]	1.655	14.276	21.205	10.577
7.51 mN (2 mL)	15.585[a]	1.001	14.508	18.662	6.421
7.24 mN (5 mL)	16.004[b]	1.367	14.077	19.628	8.540

Note: [a, b] – different superscripts in a column indicate a statistically significant difference at p <0.05.

Table 7 Influence of curd firmness (volume of rennet solution) on whey dry matter (%).

Curd firmness (Volume of rennet solution)	Average	Standard deviation	Minimum	Maximum	Coefficient of variation (%)
5.91mN (1 mL)	7.557[a]	0.174	7.230	7.886	2.304
7.51 mN (2 mL)	7.477[a]	0.109	7.310	7.712	1.454
7.24 mN (5 mL)	7.312[a]	0.109	7.105	7.548	1.497

Note: [a] – different superscripts in a column indicate a statistically significant difference at p < 0.05.

Table 5 Influence of curd firmness (volume of rennet solution) to weight of curd after 1 min dripping (g).

Curd firmness (Volume of rennet solution)	Average	Standard deviation	Minimum	Maximum	Coefficient of variation (%)
5.91mN (1 mL)	70.30[a]	5.46	60.98	79.13	7.76
7.51 mN (2 mL)	68.15[ab]	4.03	61.01	76.58	5.91
7.24 mN (5 mL)	65.52[b]	5.98	52.82	77.23	9.12

Note: [a, b] – different superscripts in a column indicate a statistically significant difference at p <0.05.

matter was a 15.645% for 5.91 mN, 15.858% for 7.51 mN and 16.004% for 7.24 mN. There was not statistically significant difference between 5.91 mN and 7.51 mN, but difference was between 5.91 mN and 7.24 mN.

In Table 7 is shown, that is not any relation between the volume of rennet solution and whey dry matter. **Djurić et al. (2004)** presented in their work that whey dry matter is 7.000%. This value is slightly lower than our results. There is any significant difference between adding 1 mL (dry matter 7.557%), 2mL (7.477%) or 5 mL (7.312%) of rennet solution to whey dry matter.

CONCLUSION

Different volume of rennet solution had influence on curd firmness. Difference of curd firmness was statistically significant between 1 mL and 2 mL, 1 mL and 5 mL of rennet solution, but difference between 2 mL and 5 mL rennet solution was not statistically significant.With a higher volume of adding rennet solution is the rennet coagulation time lower, but the highest curd firmness had the curd, which was prepared with an addition of 2 mL of rennet solution. Curd firmness (volume of rennet solution) had no significant influence to curd quality (five grade scale). All curd was classified as very good and hard, keeping its shape after its removal from the container; whey was clear with yellow-greenish colour (1) or as good as curd but a little softer, not keeping a shape quite perfectly; whey is greenish (2).

Curd firmness had an influence to a volume of whey release (mL) from the curd. With the increasing curd firmness was increase the volume of released whey from the curd. The difference between groups was statistically significant.

The statistically significant difference was between weight of curd prepared by 1mL and 5 ml of rennet solution. There is not a statistically significant difference between 1 mL and 2 mL or 2 mL and 5 mL of rennet solution. Curd dry matter was higher with a higher volume of rennet solution. Volume of added rennet solution had no statistically significant effect to change of whey dry matter.

REFERENCES

Bittante, G. 2011. Modeling rennet coagulation time and curd firmness of milk. *Journal of Dairy Science*, vol. 94, no. 12, p. 5821-5832. https://doi.org/10.3168/jds.2011-4514

Bujko, J., Kocman, R., Žitný, J., Trakovická, A., Hrnčár, C. 2011. Analyse of trans of milk production in dairy cows. *Potravinarstvo*, vol. 5, no. 1, p. 5-9. Burgess, K. J. 1978. Measurement of the Firmness of Milk Coagulum. *Irish Journal of Food Science and Technology*, vol. 2, no. 2, p. 129-134. https://dx.doi.org/10.5219/93

Czech state standart No 57 0530:1974. *Methods for testing of milk and milk products (Metody zkoušení mléka a tekutých mléčných výrobků.*

Djurić, M., Carić, M., Milanović, S., Tekić, M., Panić, P. 2004. Developmentofwhey-basedbeverages. *European Food Research and Technology*, vol. 219, no. 4, p. 321-328.

Donato, L., Guyomarc'h, F. 2009. Formation and properties of the whey protein/κ-caseincomplexes in heated skim milk – A review. *Dairy science & technology*, vol. 89, no. 1, p. 3-29. https://doi.org/10.1051/dst:2008033

EN ISO 8968-1:2002. *Milk and milk products – Determination of nitrogen content. Part 1, Kjeldahl method and crude protein calculation.*

Fox, F., McSweeney, P. L. H., Cohan, T. M., Guinee, T. P. 2004. *Cheese Chemistry, Physics and Microbiology*. 3rd ed., Oxford : Elsevier Ltd. 617 p. ISBN-13: 978-0122636523.

Fox, P. F., Guinee, T. P., Cogan, T. M., McSweeney, P. L. H. 2000. *Fundamentals of cheese science*. Maryland : AspenPublishers. 638 p. ISBN 0834212609.

ISO 2446:2008. *Milk – Determination of fat content*.

ISO 6731:2010. *Milk, cream and evaporated milk – Determination of total solids content.*

Krkošková, B. 1986. *Textúra potravín (Food texture)* . Bratislava : Alfa – Vydavateľstvo technickej a ekonomickej literatúry. 194 p.

Kuchtík, J., Šustová, K., Urban, T., Zapletal, D. 2008. Effect of the stage of lactation on milk composition, its properties and the quality of rennet curdling in East Friesian ewes. *Czech Journal of Animal Science*, vol. 53, no. 2, p. 55-63.

López, M. B., Jordán, M. J., Granados, M. V. Fernández, J. C., Castillo, M., Laencina, J. 1999. Viscosity ganges during rennet coagulation of Murciano-Granadina goat milk. *International Journal of Dairy Technolgy*, vol. 52, no. 3, p. 102-106. https://doi.org/10.1111/j.1471-0307.1999.tb02081.x

Lucey, J. A. 2002. Formation and physical properties of milk protein gels. *Journal of Dairy Science*, vol. 85, no. 2, p. 281-294. https://doi.org/10.3168/jds.S0022-0302(02)74078-2

McMahon, D. J., Brown, R. J. 1982. Evaluation of Formagraph for Comparing Rennet Solutions. *Journal of Dairy Science*, vol. 65, no. 8, p. 1639-1642. https://doi.org/10.3168/jds.S0022-0302(82)82390-4

Nájera, A. I., De Renobales, M., Barron, L. J. R. 2003. Effects of pH, temperature, $CaCl_2$ and enzyme concentrations on the rennet-clotting properties of milk: a multifactorial study. *Food Chemistry*, vol. 80, no. 3, p. 345-352. https://doi.org/10.1016/S0308-8146(02)00270-4

Payne, F. A., Hicks, C. L., Shen, P. S. 1993. Predicting optimal cutting time of coagulating milk using difuse reflectance. *Journal of Dairy Science*, vol. 76, no. 1, p. 48-61. https://doi.org/10.3168/jds.S0022-0302(93)77322-1

Pretto, D., Kaart, T., Vallas, M., Jõudu, I., Henno, M., Ancilotto, L., Cassandro, M., Pärna, E. 2011. Relationships between milk coagulation property trans analyzed with different methodologies. *Journal of Dairy Science*, vol. 94, no. 9, p. 4336-4346. https://doi.org/10.3168/jds.2011-4267 PMid:21854906

Pytel, R., Šustová, K., Kumbár, V., Nedomová, Š. 2016. A comparison of determination of the rennet coagulation properties of bovine milk. *Potravinarstvo*, vol.10, no. 1, p. 366-371. https://doi.org/10.5219/604

Roginski, H., Fuquay, J. W., Fox, P. 2003. *Encyclopedia of Dairy Sciences: Volume 1*. New York : Academic Press, 557 p. ISBN-13: 0122272358.

Sbodio, O. A., Tercero, E. J., Coutaz, R., Revelli, G. R. 2006. Effect of rennet and sodium chloride concentration on milk coagulation properties. *Ciencia y Technologia Alimentaria*, vol. 5, no. 3, p. 182-188. https://doi.org/10.1080/11358120609487690

Spreer, E. 1998. *Milk and dairy product technology*. New York : CRC Press. 483 p. ISBN 0-8247-0094-5.

Storry, J. E., Ford, G. D. 1982. Development of coagulum firmness in renneted milk – a two-phase process. *Journal of Dairy Reserch*, vol. 49, no. 2, p. 343-346. https://doi.org/10.1017/S0022029900022457

Teplý, M., Mašek, J., Havlová, J. 1976. *Syřidla živočišná a mikrobiální (Annimal and microbial rennets)*. Praha : SNTL – Nakladatelství technické literatury. 287 p.

Walstra, P., Wouters, J. T. M., Geurts, T. J. 2006. *Dairy science and technology*. 2nd ed. BocaRaton : CRC Press – Taylor & Francis. 782 p. ISBN-13:978-0-8247-2763-5.

Acknowledgments:

This work was supported by the project TP 2/2017 "Effect of aditives on the rheological behaviour on foodstuffs and raw materials for their production" financed by IGA AF MENDELU.

Contact address:

Ing. Roman Pytel, Mendel University in Brno, Faculty of AgriSciences, Department of Food Technology, Zemědělská 1, 613 00 Brno, Czech Republic, E-mail: roman.pytel@mendelu.cz

doc. Ing. Šárka Nedomová, Ph.D., Mendel University in Brno, Faculty of AgriSciences, Department of Food Technology, Zemědělská 1, 613 00 Brno, Czech Republic, E-mail: snedomov@mendelu.cz

Ing. Vojtěch Kumbár, Ph.D., Mendel University in Brno, Faculty of AgriSciences, Department of Technology and Automobile Transport, Zemědělská 1, 613 00 Brno, Czech Republic, E-mail: vojtech.kumbar@mendelu.cz

Ing. Libor Kilián, Mendel University in Brno, Faculty of AgriSciences, Department of Food Technology, Zemědělská 1, 613 00 Brno, Czech Republic, E-mail: kilian3@seznam.cz