

## Effect of Low Methoxylpectin in Acidified Milk Gels

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**Abstract:** The influence of Low Methoxyl Pectin (LMP) on the rheological and biochemical properties of milk acidified with glucono delta lactone (gdl) was investigated by measuring rheology,  $\zeta$  potential, particle size, micelle hydration. Glucono-delta-lactone (gdl) addition decreased rapidly the pH and stabilized it at 4.6-4.7. During acidification of milk reconstituted with pectin stiffness (K) increased, while the gel point decreased. The presence of pectin on milk acidified with gdl increased significantly rheological properties compared with a non treated sample. These parameters are more important when milk is reconstituted with pectin and storage at 4°C for 16 h before use. The increase of the storage temperature of milk reconstituted with pectin and storage at 4, 15 and 20°C didn't change significantly rheological properties. According to the reorganization of the casein micelles the addition of the pectin increased the particle size and the hydration of the casein micelle. The particle size of milk reconstituted with pectin continued to increase, when we increased the storage temperature of 4°C to 15-20°C. The ionic environment didn't change in the presence or in the absence of pectin.

**Key words:** Pectin casein gel, rheology, particle size, zeta potential, micelle hydration

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### INTRODUCTION

Pectin has widely used for the texture and the stabilization of the dairy products (Basak and Ramaswamy, 1994; Ramaswamy and Basak, 1992). Pectin molecules interact with caseins through calcium ions and prevent their aggregation by an ionic and steric stabilization in acidic milk drinks (Atamer *et al.*, 1999; Lucey *et al.*, 1999). According to Oakenfull and Scott (1998), the Low Methoxyl Pectin (LMP) doesn't form complex with the micelles of casein to neutral pH, where the two polymers are charged negatively. At neutral pH, where both casein micelles and pectin carry negative charges, the interaction is minimal (Oakenfull *et al.*, 1999).

When milk is acidified electrostatic repulsion between casein particles is reduced and caseins begin to aggregate as the isoelectric point is approached. The two acidification processes used in the dairy industry result in a slow reduction of pH that can require >15 h (Lucey *et al.*, 1998). In the traditional process, milk is acidified by bacteria, which ferment lactose to lactic acid. A second process that has gained the attention of food industry is direct acidification by the addition of a lactone, such as glucono-d-lactone (gdl). Gdl is an internal ester that spontaneously hydrolyzes to form gluconic acid with first-order reaction hydrolysis kinetics (De Kruif, 1997).

There have been several reports on the rheological properties of acid milk gels formed by the use of gdl (Van Slyke, 1922; Lucey *et al.*, 1997; Braga *et al.*, 2006; Raouche *et al.*, 2007).

Whereas the aggregation and gelation of casein micelles as a result of acidification have been studied frequently (Lucey and Singh, 2003) the presence of hydrocolloids during the acid induced gelation presents another degree of complexity of milk protein systems that needs to be further explored.

As pH drops during fermentation or acidification, steric and electrostatic repulsion of adjacent casein micelles is reduced and micellar aggregation-flocculation occurs (De Kruif, 1999). The use of pectin prevents casein micelle aggregation and it is widely accepted that at low (pH<5), pectin is adsorbed to casein micelles, constituting a physical barrier to aggregation (Tuimier *et al.*, 2002).

During acidification of milk demineralization of casein micelles occur and an important quantity of calcium is solubilized (Le Graet and Brule, 1993). The ionic calcium released into the serum phase during the acidification of casein micelles (Law and Leaver, 1998; Singh *et al.*, 1996) created favourable gelling conditions for the pectin and adsorption of pectin around the casein particles through electrostatic interaction seems to occur at or below

pH = 5.0 (Tuinier *et al.*, 2002) makes casein micelles and pectin an interesting biopolymer mixture that could result in different gel networks upon acidification.

The objective of this study is to define optimal conditions in order to form stable and homogeneous mixed gel with cooperative casein-pectin interactions. Milk treated with or without pectin was acidified with 1.4% (w/v) of glucono delta lactone (gdl). In comparison with non treated samples, we investigated the effect of pectin on the rheological properties of the gel obtained by acid coagulation with gdl and on micelle reorganisation. We also studied the effect of storage temperature of 4, 15 and 20°C of milk reconstituted with pectin on the rheological and biochemical properties.

## MATERIALS AND METHODS

**Milk reconstitution:** Reconstituted skim milk was obtained by dissolving a commercial non-fat low heat powder (BBA Lactalis, Bourgbarre, France) at 12% (w/w) in deionized water. To prevent bacterial growth, 0.02% (w/w) sodium azide was added. After stirring for 1 h at room temperature, pH was measured using a pH electrode (InLab-420, Mettler-Toledo, Viroflay, France) connected with a temperature compensation probe (Fisher-Bioblock Scientific, Illkrich, France) to a multi-channel analyzer (C833, Consort NV, Turnhout, Belgium). To allow complete re-hydration, milk was stored overnight at 4°C.

**Pectin supplemented:** Pectin was utilized as a stabilizer agent. Low Methoxyl Pectin (AMP 605) (SKW Biosystems, France) characterised by an esterification rate of 26-30% and an amidation rate of 17-19% was mixed to skim milk powder in order to have an optimal ratio between pectin and casein quantity. Pectin was mixed at 0.77% (w/w) to skim milk powder in order to have a link of 0.3 between pectin and casein quantity (Kouame *et al.*, 2009). The reconstitution was made with deioniser water and sodium azide like the control milk. Pectin was incorporated to milk either at the time of reconstituted milk or 1 h before acidification. After stirring for 1 h at room temperature, milk was stored at 4°C for 16 h before use or was acidified immediately.

### Gdl gelation

**Rheological measurements:** Acid coagulation of milk was achieved by addition of 1.4% (w/v) of gdl. Samples were stirred for 1 min and left for about 20 h at 30°C to obtain a final pH of 4.6. The rheological properties of the acid gels were determined one time on each sample by using a rheometer Viscoprocess (Metravib, Ecully, France)

consisting of a probe, an electronic part for signal transmission and a computer for data processing. The probe consisted of two piezoelectrical ceramics (Merzeau, 1985) arranged face to face. When an electrical tension was applied to one ceramic, its dimensions were changed: the sinusoidal electrical signal was transformed into mechanical deformation. Conversely, the piezoelectrical ceramic transformed mechanical deformations into electrical signals. If a sample (fluid, solid or viscoelastic material) was confined between two piezoelectrical ceramics, it transmitted the deformation from one to the other.

For all experiments, the probe was inserted into a thermostated beaker filled with 100 mL of milk samples. After gdl addition at the concentration of 1.4% (w/v) the milk was overlaid with a thin layer of paraffin oil to prevent drying at the surface. Measurements were started of 1 min after gdl addition. Gel formation was then monitored for 1200 min during 20 h at a frequency of 10 Hz using a viscoprocess rheometer (Metravib, Ecully, France) described by Lagoueyte *et al.* (1995). The pH was measured in parallel with a pH meter (Consort C833 multiparameter analyzer).

The stiffness  $K$ , defined (at low strain) as the stress divided by the strain was recorded as a function of time and expressed in  $\text{Nm}^{-2}$ . To evaluate the Gelation Time (GT) by the viscoprocess apparatus, we considered the point, where the stiffness became greater than the initial mean value calculated. We called this time the gel point (pg). All samples were tested in triplicate.

**Separation of colloidal and soluble phases:** Separation of the soluble and the micellar phases of milk was achieved by centrifugation of milk samples at 149,000x g for 55 min at 20°C with a Beckman L 7-65 ultracentrifuge, rotor 70Ti (Beckman Instruments France S.A., Gagny, France). The soluble phase including the supernatant and the opalescent layer was removed, leaving a firm pellet considered as the colloidal phase. In all analysis, the composition of this phase was calculated as the difference between the amount of the components in the total milk and in the soluble phase.

**Hydration of casein micelles:** The pellet of each sample was freeze dried for 48 h and oven dried at 102°C for 1 h. Six trials were performed on each sample. The levels of hydration of casein micelles were calculated according to the formula of Thompson *et al.* (1969):

$$\text{Micelle hydration (g H}_2\text{Og}^{-1} \text{ of sedimented protein)} = \frac{\text{Wet pellet (g)} - \text{Dry pellet (g)}}{\text{Dry pellet}}$$

**Statistical analysis:** All results obtained were analyzed using statistica version 7.1 (StarSoft, Inc., Tulsa, Ok, USA). In order to determine the significance of the different experimental factors on all evaluators, the Analysis of Variance (ANOVA) at a confidence level of 95% was performed. To test significant differences between means. Student t-test was used at a 5% significant level.

**RESULTS AND DISCUSSION**

**Acid gelation:** The mechanical properties were determined using a non destructive dynamic measurement described by Lagoueyte *et al.* (1995).

The decreased of pH and changes in the stiffness (K) of milk gels as a function of time after gdl addition to milk with or without pectin are shown in Fig. 1-3. The values of the gel point (Pg), stiffness at 20 h after gdl addition (Kmax) of milk are shown in Table 1. A decrease of Pg and an increase of Kmax were observed for pectin supplemented milks.

During the milk storage, the casein micelles of control milk and pectin supplemented milk are very stable in native milk conditions (Walstra, 1990) at pH 6.64±0.02 and 6.64±0.01 (Fig. 1). According to Harte *et al.* (2007), at pH of milk, κ-casein acts as an electrostatic and physical barrier toward aggregation of adjacent casein micelles (De Kruijff, 1999) and Low Methoxyl Pectin remains dispersed in the serum phase.

The presence of gdl decreased rapidly the pH of milk. According to De Kruijff (1997), gdl was rapidly hydrolysed to gluconic acid, resulting in a rapid reduction in pH initially.

After a constant stage, stiffness (K) increased very fast in all samples (Fig. 2). The abrupt change in the slope observed between pH 4.9 and 5.05, respectively for control milk and milk with pectin (Fig. 3) corresponded to the sol gel transition of milk and indicated that the onset of gelation took place between this range of pH. Mechanisms involved in milk gelation upon acidification are not well established. But Banon and Hardy (1992) have described the acid gelation process with 3 stages: collapse of the κ-casein hairy layer; casein micelle aggregation and gel reticulation.

The results are in good agreement with those of Gastaldi *et al.* (1996, 1997). Roefs *et al.* (1990) and Lucey *et al.* (1997) attributed the increase in stiffness of gels to increase fusion of particles and clusters due to rearrangement of both inter and intra molecule.

The gel point of control milk was 400±5 min at pH 4.9±0.04. These results are in accordance with previous studies (Lucey *et al.*, 1997; Vasbinder *et al.*, 2003;

Table 1: Effect of pectin on rheological parameters (gel point (Pg), Kmax, pH) of milk supplemented or not with pectin

Parameters	Control milk	Milk supplemented with pectin (4°C)	
		Before storage	After storage
Pg (min)	400±5	231±4	263±3
Kmax (N m <sup>-1</sup> )	8580	33800	26400
pHi	6.64±0.02	6.61	6.61
pHg	4.95±0.04	5.05	5.05
pHf	4.6±0.02	4.6	4.6

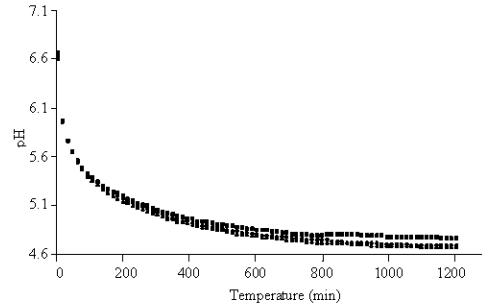


Fig. 1: pH as a function of time; control milk (■), milk with pectin incorporated and storage at 4°C (◆) and milk with pectin incorporated 1 h before acidification (▲)

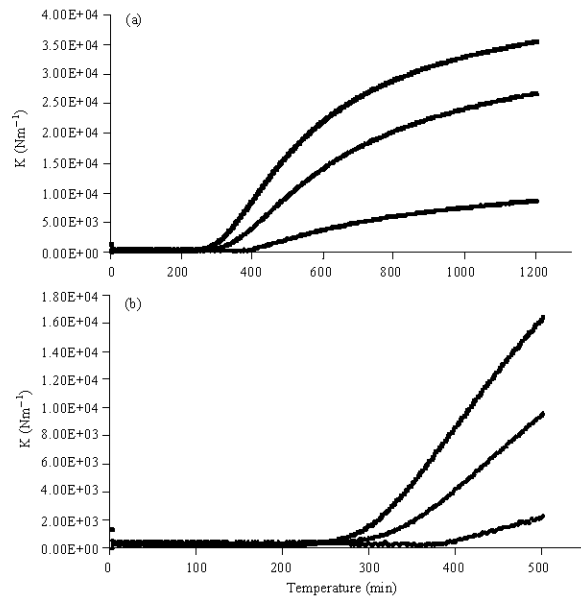


Fig. 2: Stiffness (K) as a function of time; control milk (■), milk with pectin incorporated and storage at 4°C (◆), milk with pectin incorporated 1 h before acidification (▲) (a) 20 h after gdl addition and (b) During the first 500 min after gdl addition

Raouche *et al.*, 2007). As pH decreases, calcium ions migrate from the casein micelle to the serum phase and promote crosslinking of LM pectin chains (Harte *et al.*, 2007). So, when milk was supplemented with pectin the

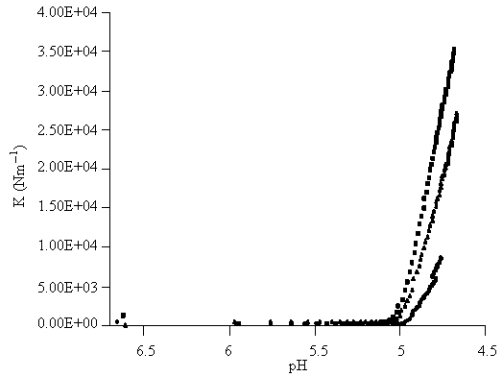


Fig. 3: Stiffness (K) as a function of pH during gdl gelation at 30°C of control milk (■), milk with pectin incorporated and storage at 4°C (◆) and milk with pectin incorporated 1 h before acidification at 30°C (▲)

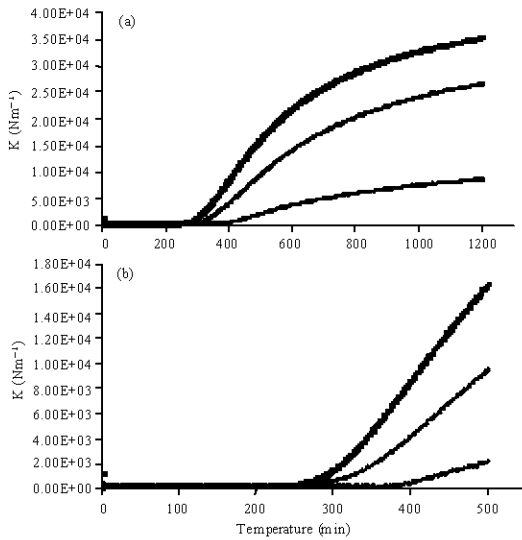


Fig. 4: Stiffness (K) as a function of time during gdl gelation at 30°C of milk supplemented with pectin and storage at 4°C (◆) at 15°C (◆) and at 20°C (◆) and acidified with gdl at 30°C (a) 20 h after gdl addition and (b) During the first 500 min after gdl addition

gel point decreased significantly at pH 5.05 and was 263±3 and 231±4, respectively for milk supplemented with pectin 1 h before acidification or after storage for 16 h at 4°C. Harte *et al.* (2007) characterized this pH at a pectin-pectin interaction induced by Ca<sup>2+</sup> release from the casein micelle. When pH was dropped in the 5.0-4.6 the increased of stiffness in this range of pH (Fig. 3) is characterized by adsorption of low methoxyl pectin onto casein micelle (Harte *et al.*, 2007). According to these researchers near pH 5.0, micelles are depleted of ionic calcium, the κ-casein brush collapses and maximized

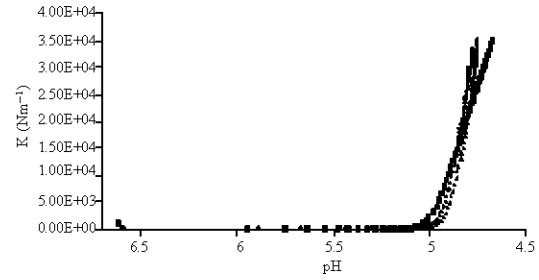


Fig. 5: Stiffness (K) as a function of pH during gdl gelation at 30°C of milk supplemented with pectin and storage at 4°C (◆), at 15°C (◆) and at 20°C (◆) and acidified with gdl at 30°C

Table 2: Effect of storage temperature on rheological parameters (gel point (Pg), Kmax, pH) of milk supplemented with pectin

Parameters	Milk supplemented with pectin and storage		
	4°C	15°C	20°C
Pg (mm)	231±3	227±8,5	222±8
Kmax (Nm <sup>-1</sup> )	33800	33067	36266
pHi	6.64±0.01	6.64±0.01	6.64±0.01
pHg	5.05	5.06	5.05
pHf	4.6	4.7	4.7

Table 3: Effect of storage temperature on some micellar characteristics of milk supplemented with pectin

Micellar characteristics	Control milk	Milk supplemented with pectin and storage		
		4°C	15°C	20°C
Particle size	230±3	256±3	348±2	350±3
Zeta potential	-30±3	-29±3	-28±3	-29±3
Hydration of micelle	2.65±0.1	4.41±0.15	6.54	6.51

calcium-induced LM pectin aggregation occurs. A further drop in pH to 4.6 promotes the isoelectric aggregation of casein micelles and contributes to the formation of casein-pectin complexes.

The final stiffness (K) of the gels treated with or without pectin was significantly different. Stiffness of milk supplemented with pectin increased significantly. These results are in accordance with previous studies (Matia *et al.*, 2004). These researchers observed an increased of storage modulus with an increasing of pectin concentration for 1.0% pectin and a decrease of the gel point with increasing pectin content from 1.5 h (at 0.02%) to 16 min (at 1.0%) following addition of the gdl.

The rheological properties of milk reconstituted with pectin and storage at 15 and 20°C are shown in Fig. 4 and 5. The final stiffness (K) of the gels was not different significantly (Table 2).

**Casein micelle characterisation:** The characteristic micelles of the control milk are presented in Table 3. The average size of 230 nm, the zeta potential of -29 mV and the water solvation of 2.65 g. H<sub>2</sub>O g<sup>-1</sup> of sedimented

protein are in accordance with previous studies (Guillaume *et al.*, 2004; Gastaldi *et al.*, 1997; Wade *et al.*, 1996). The addition of pectin to control milk had a significant effect on those two parameters: the average size and the micelle hydration rose to 256 nm and 4.41 g. H<sub>2</sub>O g<sup>-1</sup> of protein, respectively (Table 3).

The presence of pectin increased significantly these parameters. Nakamura *et al.* (2006) found an increase in the particle diameter of skim milk with increasing pectin concentration in non acidified milk. These researchers indicated an association of pectin to the casein micelles at the initial pH, possibly because of the presence of calcium ions.

### CONCLUSION

This research showed that low methoxyl pectin has a large influence on the rheological of acid induced caseinate gel. While, the presence of pectin increased the particle size and the hydration of casein, the zeta potential not changed. During the acidification of milk, the increase in stiffness of the gel in the presence of pectin showed that pectin with his gelling function improved the rheological properties like gel point and stiffness of milk.

### REFERENCES

Atamer, M., A. Gursel, B. Tamucay, N. Gencer, G. Ytldirum, S. Odabasi, E. Karademir, E. Senel and S. Kirdar, 1999. A study on the utilization of pectin in manufacture of long-life ayran. *Gida*, 24 (2): 119-126. <http://www.gidadernegi.org/EN/Genel/BelgeGoster.aspx?17A16AE30572D313AAF6AA849816B2EF3F719885B09FDCBF>.

Banon, S. and J. Hardy, 1992. A colloidal approach of milk acidification by glucono-delta-lactone. *J. Dairy Sci.*, 75: 935-941. <http://jds.fass.org/cgi/reprint/75/4/935.pdf>.

Basak, S. and H.S. Ramaswamy, 1994. Simultaneous evaluation of shear rate and time dependency of stirred yogurt rheology as influenced by added pectin and strawberry concentrate. *J. Food Eng.*, 21: 385-393. DOI: 10.1016/0260-8774(94)90081-7.

Braga, A.L.M., M. Menossi and R.L. Cunha, 2006. The effect of the glucono-delta-lactone/caseinate ratio on sodium caseinate gelation. *Int. Dairy J.*, 16 (5): 389-398. DOI: 10.1016/j.idairyj.2005.06.001.

De Kruif, C.G., 1997. Skim milk acidification. *J. Colloid Interface Sci.*, 185: 19-25. <http://www.ingentaconnect.com/content/ap/cs/1997/00000185/00000001/art04548>.

De Kruif, C.G., 1999. Casein micelle interactions. *Int. Dairy J.*, 9 (3): 183-188.

Gastaldi, E., A. Lagaude and B. Tarodo De La Fuente, 1996. Micellar transition state in casein between pH 5.5 and 5.0. *J. Food Sci.*, 61: 59-64, 68. DOI: 10.1111/j.1365-2621.1996.tb14725.x.

Gastaldi, E., A. Lagaude, S. Marchesseau and B. Tarodo De La Fuente, 1997. Acid milk gel formation as affected by total solids content. *J. Food Sci.*, 62 (4): 671-687. DOI: 10.1111/j.1365-2621.1997.tb15432.x.

Guillaume, C., L. Jimenez, J.L. Cuq and S. Marchesseau, 2004. An original pH-reversible treatment of milk to improve rennet gelation. *Int. Dairy J.*, 14 (4): 305-311. DOI: 10.1016/S0958-6946(03)00149-3.

Harte, F.M., I.C. Montes, M. Adams and M.F. San Martin Gonzalez, 2007. Solubilized micellar calcium induced Low Methoxyl-Pectin aggregation during milk acidification. *J. Dairy Sci.*, 90: 2705-2709. DOI: 10.3168/jds.2006-629.

Kouame, A.F., A.H. Klandar, S. Marchesseau and A. Lagaude, 2009. Effect of a CO<sub>2</sub> acidification cycle on physicochemical and acid gelation properties of skim milk reconstituted with added pectin. *Int. Dairy J.*, 19: 393-398. DOI: 10.1016/j.idairyj.2009.02.002.

Lagoueyte, N., A. Lagaude and B. Tarodo De La Fuente, 1995. Rheological properties of rennetd reconstituted milk gels by piezoelectric viscoprocess: Effects of temperature and calcium phosphate. *J. Food Sci.*, 60 (6): 1344-1348, 1363. DOI: 10.1111/j.1365-2621.1995.tb04588.x.

Law, A.J.R. and J. Leaver, 1998. Effects of acidification and storage of milk on dissociation of bovine casein micelles. *J. Agric. Food Chem.*, 46: 5008-5016. <http://pubs.acs.org/doi/abs/10.1021/jf980748p>.

Le Graet, Y. and G. Brule, 1993. Effects of pH and ionic strength on distribution of mineral salts in milk. *Lait*, 73: 51-60. DOI: 10.1051/lait:199312.

Lucey, J.A., T. Van Vliet, K. Grolle, T. Geurts and P. Walstra, 1997. Properties of acid casein gels made by acidification with glucono-δ-lactone. 2. Syneresis, permeability and microstructural properties. *Int. Dairy J.*, 7: 389-397. <http://linkinghub.elsevier.com/retrieve/pii/S0958694697000277>.

Lucey, J.A., M. Tamehana, H. Singh and P.A. Munro, 1998. Effect of interactions between denatured whey proteins and casein micelles on the formation and rheological properties of acid skim milk gels. *J. Dairy Res.*, 65: 555-567. DOI: 10.1017/S0022029998003057.

Lucey, J.A., M. Tamehana, H. Singh and P.A. Munro, 1999. Stability of model acid milk beverage: Effect of pectin concentration, storage temperature and milk heat treatment. *J. Texture Stud.*, 30: 305-318. DOI: 10.1111/j.1745-4603.1999.tb00219.x.

- Lucey, J.A. and H. Singh, 2003. Acid Coagulation of Milk. In: Fox, P.F. and P.L.H. McSweeney (Eds.), *Advanced Dairy Chemistry*. Kluwer Academic/Plenum. and Singh, London. Proteins, 1: 1001-1025. <http://riddet.massey.ac.nz/scientific-publications-2003.php>.
- Matia, M.L., K. Lau and E. Dickinson, 2004. Effects of low-methoxyl amidated pectin and ionic calcium on rheology and microstructure of acid-induced sodium caseinate gels. *Food Hydrocoll.*, 18: 271-281. DOI: 10.1016/S0268-005X(03)00083-3.
- Merzeau, P., 1985. Appareil pour la mesure des proprietes physiques d'un milieu, notamment les proprietes rheologiques. Brevet n°8515441.
- Nakamura, A., R. Yoshida, H. Maeda and M. Corredig, 2006. The stabilizing behaviour of soybean soluble polysaccharide and pectin in acidified milk beverages. *Int. Dairy J.*, 16(4): 361-369. DOI: 10.1016/j.dairyj.2005.01.014.
- Oakenfull, D. and A. Scott, 1998. Milk Gels with Low Methoxyl Pectins. In: Williams, P.A. and G.O. Phillips (Eds.). *Gums and Stabilizers for the Food Industry*. Cambridge, UK. Royal Soc. Chem., 9: 212-221. <http://linkinghub.elsevier.com/retrieve/pii/S0268005X03000833>.
- Oakenfull, D., E. Miyoshi, K. Nishinari and A. Scott, 1999. Rheological and thermal properties of milk gels formed with kappa-carrageenan. I. Sodium caseinate. *Food Hydrocolloids*, 13 (6): 525-533.
- Raouche, S., M. Dobenesque, A. Biot, A. Lagaude, J.L. Cuq and S. Marchesseau, 2007. Stability of casein micelle subjected to reversible CO<sub>2</sub> acidification: Impact of holding time and chilled storage. *Int. Dairy J.*, 17 (8): 873-880. DOI: 10.1016/j.dairyj.2006.11.006.
- Ramaswamy, S. and S. Basak, 1992. Pectin and raspberry concentrate effects on the rheology of stirred commercial yogurt. *J. Food Sci.*, 57 (2): 357-360. DOI: 10.1111/j.1365-2621.1992.tb05494.x.
- Roefs, S.P.F.M., A.E.A. De Groot-Mostert and T. Van Vliet, 1990. Structure of acid casein gels. I. Formation and model of gel network. *Colloids and Surfaces*, 50: 141-159. DOI: 10.1016/0166-6622(90)80259-7.
- Singh, H., M.S. Roberts, P.A. Munro and C.T. Teo, 1996. Acid-induced dissociation of casein micelles in milk: Effects of heat treatment. *J. Dairy Sci.*, 79 (8): 1340-1346. <http://jds.fass.org/cgi/reprint/79/8/1340.pdf>.
- Thompson, M.P., R.T. Boswell, V. Martin, R. Jenness and C.A. Kiddy, 1969. Casein-pellet-solvation and heat stability of individual cow's milk. *J. Dairy Sci.*, 52: 796-798. <http://jds.fass.org/cgi/content/abstract/52/6/796>.
- Tuinier, R., C. Rolin and C.G. De Kruif, 2002. Electrosorption of pectin onto casein micelles. *Biomacromol.*, 3 (3): 632-638. DOI: 10.1021/bm-025530x.
- Van Slyke, D.D., 1922. On the measurement of buffer values and on the relationship of buffer value to the dissociation constant buffer and the concentration and reaction of buffer solution. *J. Biol. Chem.*, 52: 525-571. <http://www.jbc.org/content/52/2/525.full.pdf+html>.
- Vasbinder, A.J., A.C. Alting, R.W. Visschers and C.G. De Kruif, 2003. Texture of acid milk gels: Formation of disulfide cross-links during acidification. *Int. Dairy J.*, 13: 29-38.
- Wade, T., J.K. Beattie, W.N. Rowlands and M.A. Augustin, 1996. Electroacoustic determination of size and zeta potential of casein micelles in skim milk. *J. Dairy Res.*, 63: 387-404. [http://d.wanfangdata.com.cn/NSTLQK\\_NSTL\\_QK9892326.aspx](http://d.wanfangdata.com.cn/NSTLQK_NSTL_QK9892326.aspx).
- Walstra, P., 1990. On the stability of casein micelles. *J. Dairy Sci.*, 73: 1965-1979. <http://www.jbc.org/content/52/2/525.full.pdf>.