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Dairy Sci. & Technol. (2011) 91:383–395
DOI 10.1007/s13594-011-0004-y

NOTE

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Received: 30 January 2010 / Revised: 25 September 2010 / Accepted: 29 November 2010 /
Published online: 10 March 2011

Abstract A comparison of rennet coagulation of milk supplemented with vacuum-condensed or ultrafiltered milk was undertaken. Five treatments with two levels of protein (45 and 60 $g \cdot kg^{-1}$) from vacuum-condensed (CM1 and CM2) and ultrafiltered milk (UF1 and UF2) along with a 32 $g \cdot kg^{-1}$ protein control were compared. A Formagraph was used to characterize rennet clotting time (RCT), amplitude at 40 min (a_{40}), and time to achieve amplitude of 20 mm (k_{20}). Rennet was added at 192 $\mu L \cdot kg^{-1}$ milk, without prior addition of starter. RCT was lower for CM than for UF, and lower for higher protein milks. The k_{20} values were lower for higher protein samples, indicating a higher rate of curd firming; and were not affected by the method of concentration. The a_{40} values (i.e., curd firmness) were higher for samples with higher protein content. Statistically there was no effect of method of concentration on a_{40}; however subjective analysis of curds during cheese making indicated that CM curds were firmer than UF curds. T_0 further evaluate the discrepancy in coagulum characteristics, viscosity during coagulation was monitored. Samples of milk were inoculated with starter culture; each treatment was inoculated with two levels of rennet (132 and 192 $\mu L \cdot kg^{-1}$); and changes in viscosity were determined throughout coagulation using Brookfield viscometer. Calculated coagulum strength varied from 2.72 to 9.22 N·m; and increased as protein level increased. Also, CM curd was firmer than its UF counterpart. A higher rate of inoculation of rennet can reduce coagulum strength; however the rate should be optimized to avoid decreasing coagulation time to an unacceptable level.

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摘要 真空浓缩和超滤浓缩乳的酶凝乳特性

摘要 本文比较了经真空浓缩和超滤浓缩的两种浓缩乳的酶凝乳特性。选用5组样品, 其中2组经真空浓缩至蛋白质浓度分别 45g.kg^{-1}(CM1) 和 60g.kg^{-1}(CM2), 2 组超滤浓缩乳 (UF1和 UF2) 以及一组对照组 (蛋白质浓度为32 g.kg^{-1})。根据在 40min 时的振幅 (a_{40}) 以及达到振幅为 20mm 时的时间 (k_{20}) 来描述酶的凝乳时间 (RCT) 。在未加发酵剂的乳中凝乳酶的添加量为192 μL.kg^{-1}。真空浓缩乳的酶凝乳时间低于超滤浓缩乳，而且是蛋白质浓度越高，凝乳时间越短。高浓度蛋白质样品的k20 较低，表明凝块变硬的速度较高，而且浓缩方法对其影响不显著。样品蛋白质浓度越高，形成凝块的硬度越高。样品的蛋白质浓度越高，a_{40}值 (凝块的硬度) 越高。浓缩方法对 a_{40}值的影响没有统计学意义。基于对干酪制作过程中凝块的分析表明真空浓缩乳的凝块比超滤浓缩乳形成的凝块硬。本文还进一步评价了凝乳的凝聚特性和凝块形成过程中粘度变化。在接种发酵剂的乳样品中，每一处理组分别加入 2个浓度的凝乳酶(132μL.kg^{-1} 和192 μL.kg^{-1}), 采用 Brookfield 粘度仪测定了整个凝聚过程中粘度的变化。随着乳中蛋白质浓度的增加，凝聚物强度的变化范围在 2.72~9.22 Nm。同样，真空浓缩乳形成的凝块比超滤浓缩乳形成的凝块硬。较高用量的凝乳酶会降低凝块的强度，然而，通过优化凝乳过程可以避免过短的凝乳时间。

Keywords Vacuum condensing · Ultrafiltration · Rennet coagulation · Viscosity · Firmness

关键词 真空浓缩 · 超滤 · 酶凝乳 · 粘度 · 硬度

Abbreviation key

UF	Ultrafiltered milk
CM	Condensed milk
C	Control unconcentrated milk (32 g·kg^{-1} protein)
UF1	Ultrafiltered milk (45 g·kg^{-1} protein)
UF2	Ultrafiltered milk (60 g·kg^{-1} protein)
CM1	Condensed milk (45 g·kg^{-1} protein)
CM2	Condensed milk (60 g·kg^{-1} protein)
RCT	Rennet clotting time
k_{20}	Time taken for amplitude on Formagraph to reach 20 mm after rennet clotting time
a_{40}	Amplitude on Formagraph after 40 min of rennet addition
η_0	Apparent viscosity after 1 min of addition of rennet
T_0	Time at the onset of increase in apparent viscosity
T_{max}	Time when maximum apparent viscosity was reached
η_{max}	Maximum apparent viscosity
IMCU	International Milk Clotting Units

1 Introduction

Coagulation of milk by rennet is a three-stage process that involves proteolysis, aggregation, and gelation (McMahon and Brown 1984). In the first stage, rennet hydrolyses κ-casein and destabilizes the casein micelles. In the subsequent stage, the

destabilized micelles aggregate in the presence of Ca ions. These two stages are not quite distinct, viz., it is not necessary for all of the κ-casein to be hydrolyzed before aggregation of the rennet-altered micelles can take place. Hence, after a certain level of hydrolysis, both stages occur simultaneously, leading to formation of a gel that undergoes a period of curd firming and then syneresis.

Rennet clotting properties depend on the three underlying phenomena, i.e., rate of enzymatic action that follows Michaelis–Menten mechanism, degree of hydrolysis of κ-casein sufficient enough for gel formation, and rate of aggregation of destabilized casein micelles as governed by Von Smoluchowski aggregation (Garnot and Corre 1980). These phenomena are affected by a number of factors, such as, temperature (Kowalchyk and Olson 1977), pH (Cheryan et al. 1975), heat treatment of milk (Wilson and Wheelock 1972), presence of divalent cations (Lucey and Fox 1993), protein concentration of milk (Garnot and Corre 1980), and milk concentration (Culioli and Sherman 1978; Kameswaran and Smith 1999; Mehaia and El-Khadragy 1998).

Concentrating milk prior to cheese making is attractive because less bulk has to be handled to produce the same quantity of cheese. Two common methods of producing concentrated milk are ultrafiltration and vacuum condensing. Milk concentrates are often blended with unconcentrated milk to get a desired concentration ratio. Blends have higher total solids and protein contents, and in some cases, an increased ratio of protein to total solids as compared with the original milk. These differences may lead to alteration in the rennet coagulation properties of these concentrated milks. Formation of a good quality coagulum, in part, determines the quality of the final cheese produced. It is also important to cut the curd at an appropriate firmness so that syneresis takes place properly and loss of milk solids in the whey can be minimized (Bynum and Olson 1982).

A number of studies have been reported which discuss various aspects of coagulation in ultrafiltered milk (Culioli and Sherman 1978; Kameswaran and Smith 1999; Mehaia and El-Khadragy 1998). In spite of the interest of cheese manufacturers in using vacuum-condensed milk concentrates (Sandfort 1983), a little has been studied evaluating rennet coagulation behavior of vacuum-condensed and its comparison to ultrafiltered milk. Controlled experiments evaluating cheese milk supplemented with these milk concentrates will help cheese makers in troubleshooting and modulating cheese-making parameters to produce acceptable coagulum. Different analytical techniques have been proposed to characterize rennet coagulation of milk (Lucey 2002). Each of these methods measure distinct properties, destructively or non-destructively, during coagulation by using respective principles. Though the viscometric methods do not give the same results as the non-destructive rheological methods because of lack of consideration of syneresis, such methods have been suggested to complement quite well to the studies on rennet coagulation of milk retentates obtained by concentrating milk (Korolczuk and Maubois 1987).

2 Materials and methods

2.1 Experimental design

Rennet curd formation in milk samples that differed in method of concentration and protein levels were characterized. The treatments/milk samples were prepared by blending

pasteurized fat-reduced milk, cream, and ultrafiltered (UF) or vacuum-condensed (CM) milk concentrated to different protein levels. The five treatments included: fat-reduced milk and cream blended to a protein content of 32 g·kg^{-1} (C); fat-reduced milk, cream, and CM blended to a protein content of 45 and 60 g·kg^{-1} (CM1 and CM2, respectively); fat-reduced milk, cream, and UF blended to a protein content of 45 and 60 g·kg^{-1} (UF1 and UF2, respectively). All five treatments (C, CM1, UF1, CM2, and UF2) were standardized to a casein-to-fat ratio (C/F) of 0.7.

The rennet curd formation was studied using Formagraph (Experiment 1) and Brookfield viscometer (Experiment 2). For each experiment, a fresh set of milk samples (C, CM1, UF1, CM2, and UF2) were prepared. In experiment 1, six replications of milk samples were inoculated at a specific rennet concentration and analyzed for rennet coagulation parameters using Formagraph. In experiment 2, another set of four replications of the five treatments were manufactured and analyzed for changes in viscosity during coagulation, using Brookfield viscometer, at two different inoculation levels of rennet.

2.2 Milk preparation and composition

A detailed description of the milk-processing protocols that were followed to obtain the five treatments was described previously (Acharya and Mistry 2004). Samples of milk were analyzed for total solids, fat, protein, ash, Ca, and pH; and casein was estimated as 75% of the protein. Details of analytical procedures have also been described previously (Acharya and Mistry 2004). Average composition of the standardized milk samples used in experiment 1 has been previously published (Acharya and Mistry 2004), and for experiment 2 is presented in Table 1. For the two experiments, the composition of the corresponding treatments and the statistical significance of factors affecting the composition were similar.

Table 1 Composition of milk samples used for viscosity study

Parameter	Treatment				
	C	CM1	UF1	CM2	UF2
Total solids (g·kg^{-1})	122 a	174 c	151 b	221 e	177 d
Fat (g·kg^{-1})	37 a	48 b	48 b	64 c	64 c
Protein (g·kg^{-1})	34 a	45 b	45 b	60 c	60 c
Casein (g·kg^{-1})	26 a	34 b	34 b	45 c	45 c
Casein/Fat	0.70	0.71	0.71	0.70	0.71
Ash (g·kg^{-1})	7.1 a	9.8 d	8.0 b	12.0 e	9.1 c
Calcium (g·kg^{-1})	1.1 a	1.4 b	1.4 b	1.9 d	1.7 c
pH	6.73 c	6.65 a, b	6.70 b, c	6.60 a	6.70 b, c

Means in rows with same letters (a–e) do not differ ($P>0.05$). Mean of four replicates

C control (unconcentrated milk (32 g·kg^{-1} protein)), *CM1* condensed milk (45 g·kg^{-1} protein), *UF1* ultrafiltered milk (45 g·kg^{-1} protein)), *CM2* condensed milk (60 g·kg^{-1} protein), *UF2* ultrafiltered milk (60 g·kg^{-1} protein)

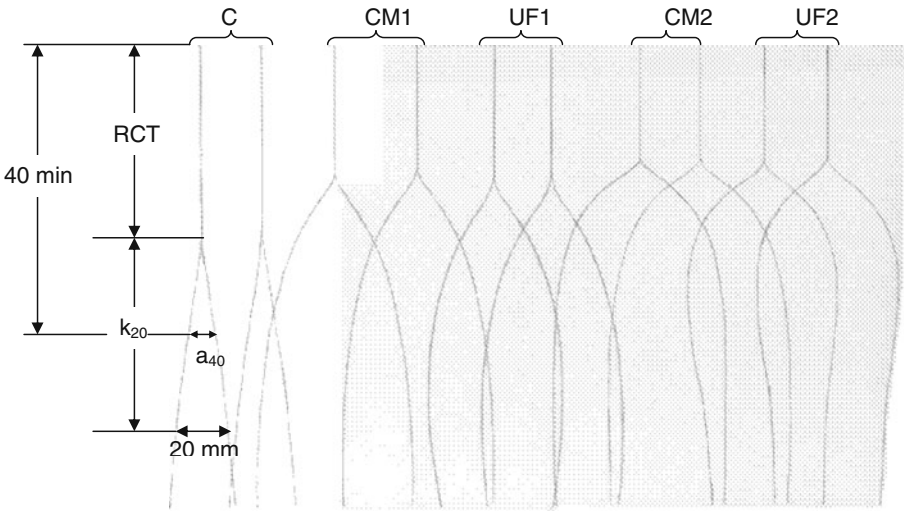

Fig. 1 Formagraph chart representing the rennet coagulation parameters of the five treatments (C, CM1, UF1, CM2, and UF2). *RCT* time from rennet addition until formation of a gel, k_{20} time from RCT until an amplitude of 20 mm had been reached, a_{40} amplitude measured 40 min after RCT

2.3 Rennet coagulation parameters using Formagraph

The rennet coagulation properties of the milk samples (C, CM1, UF1, CM2, and UF2) were analyzed in duplicate using a Formagraph® (Model 11720; N Foss Electric Hillerod, Denmark), with 2 mm/min chart speed of a light-sensitive paper (McMahon and Brown 1982). A 10-mL of sample was added to each test well of the instrument and warmed and equilibrated at 30 °C for 30 min. T_0 of these samples in the test well, 220 μL of single-strength (586 IMCU/mL) rennet (Chymostar Classic M9017, Danisco (previously Rhodia Inc.), Madison, WI), freshly diluted 1:100 was added and mixed well. This concentration of rennet corresponds to a level of 198 $\mu L \cdot kg^{-1}$ of milk. The parameters examined included: rennet coagulation time (RCT), determined as the time from addition of rennet until the two lines diverge; a_{40}, determined as the amplitude measured at 40 min after RCT; and k_{20}, the time from RCT until an amplitude of 20 mm had been reached (Fig. 1).

2.4 Viscosity changes during rennet curd formation using Brookfield viscometer

A cheddar cheese-making protocol for milk ripening was used. Milk samples (C, CM1, UF1, CM2, and UF2) were inoculated with a frozen, concentrated Direct Vat set culture (Super Start M61, Danisco (previously Rhodia Inc.), Madison, WI) at 70 $g \cdot kg^{-1}$ of protein in cheese milk. A beaker containing 200 mL of inoculated milk was incubated at 32 °C for 30 min. Subsequently, each treatment was inoculated with single-strength (586 IMCU/mL) rennet (Chymostar Classic M9017, Danisco (previously Rhodia Inc.), Madison, WI) at two different levels (i.e., 132 or 198 $\mu L \cdot kg^{-1}$ of milk) and analyzed using Brookfield RV DV-III Rheometer with a UL adapter (Model DV-III, Brookfield Engineering Labs, Stoughton, MA). The UL adapter was immersed without bottom cap in the beaker containing 200 mL of milk, which was placed in a water bath at 32 °C. Spindle was

programmed to begin at 50 rpm and increase speed by 20 rpm every 5 s until 250 rpm was reached. Speed was maintained for 40 min then stepped down by 50 rpm every 5 s until a speed of 50 rpm was reached and the viscometer was then stopped. The purpose of the step up and down was to prevent jerky start up and shut down.

During the experiment, the friction of the liquid produced a torque on the spindle proportional to the viscosity of the liquid; and the apparent viscosity was recorded every 5 s using manufacturer-supplied software (Rheocalc, Brookfield Engineering Labs, Stoughton, MA). The apparent viscosity curve was used to obtain viscosity of the liquid after 1 min (η_0, mPa·s); time at the onset of increase in viscosity (T_0, min); time when maximum viscosity was reached (T_{max}, min); and maximum viscosity obtained (η_{max}, mPa·s) (Fig. 2). These data were used to characterize gel formation and calculate coagulum strength (N·m) (Anonymous 2004). The calculated coagulum strength does not account for syneresis that take place during such measurements. In order to mitigate the confounding influence of syneresis, torque measurements, which were used for calculations, were limited until T_{max}. Effect of syneresis on measured torque is considerably larger thereafter. The calculated coagulum strength may not represent absolute coagulum strengths; hence, will be used to discuss the relative differences between the treatments.

$$\begin{aligned}\text{Coagulum strength} &\equiv \text{total energy required to break the coagulum} \\ &= T_{(\theta)} \cdot \mathrm{d}\theta \text{ from } \theta_0 \text{ to } \theta_{max}\end{aligned} \tag{1}$$

where,

$\mathrm{d}\theta$ Incremental rotational displacement
$T_{(\theta)}$ Torque (a function of displacement)
θ_0 Rotational displacement at T_0
θ_{max} Rotational displacement at T_{max}

For a cylindrical geometry,

$$\begin{aligned}T_{(\theta)} &= \left(\tau_{(\theta)} \cdot J\right)/R_b \\ &= \left(\tau_{(\theta)} \cdot \pi \cdot {R_b}^4\right)/R_b \\ &= \tau_{(\theta)} \cdot \pi \cdot {R_b}^3\end{aligned} \tag{2}$$

where,

$\tau_{(\theta)}$ Shear stress (a function of displacement)
J Polar moment of inertia=$(\pi \cdot {R_b}^4)$

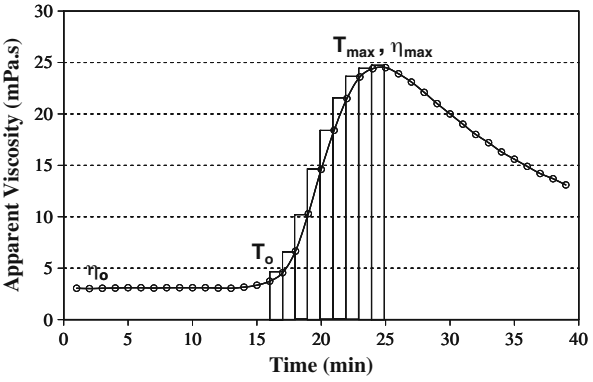

Fig. 2 Viscosity changes over time as measured using Brookfield viscometer (η_0 apparent viscosity after 1 min of addition of rennet, T_0 time at the onset of increase in apparent viscosity, T_{max} time when maximum apparent viscosity was reached, η_{max} maximum apparent viscosity. Area under the curve from T_0 to T_{max} was used to estimate coagulum strength)

R_b Radius of cylinder used for measuring viscosity

Combining Eqs. 1 and 2:

$$\begin{aligned}\text{Coagulum strength} &\equiv \text{total energy required to break the coagulum}\\ &= \pi \cdot {R_b}^3 \int \tau_{(\theta)} \cdot \mathrm{d}\theta \text{ from } \theta_0 \text{ to } \theta_{max}\end{aligned}$$

The equation can be further deduced into time units:

$$\begin{aligned}\text{Coagulum strength} &\equiv \text{total work done to break the coagulum}\\ &= \pi \cdot {R_b}^3 \int \tau_{(t)} \cdot \omega \cdot \mathrm{d}t \text{ from } T_0 \text{ to } T_{max} \ldots\ldots\ldots (\text{since } \mathrm{d}\theta = \omega \cdot \mathrm{d}t)\\ &= \pi \cdot {R_b}^3 \int \gamma \cdot \eta_{(t)} \cdot \omega \cdot \mathrm{d}t \ldots\ldots\ldots\ldots\ldots \left(\text{since } \tau_{(t)} = \gamma.\eta_{(t)}\right)\\ &= \pi \cdot {R_b}^3 \int \left(2\omega \cdot {R_c}^2/\left({R_c}^2 - {R_b}^2\right)\right) \cdot \eta_{(t)} \cdot \omega \cdot \mathrm{d}t \ldots\ldots\ldots\ldots\\ &\qquad \ldots\ldots\ldots\ldots\ldots (\text{since } \gamma = 2\omega \cdot \mathbf{R_c^2}/\left(\mathbf{R_c^2} - \mathbf{R_b^2}\right)\\ &= \left(\pi \cdot {R_b}^3\right) \cdot \left(\left(2\omega \cdot {R_c}^2/\left({R_c}^2 - {R_b}^2\right)\right) \cdot \omega\right) \cdot \int \eta_{(t)} \cdot \mathrm{d}t\\ &= \left(\pi \cdot {R_b}^3\right) \cdot \left(\left(2\omega \cdot {R_c}^2/\left({R_c}^2 - {R_b}^2\right)\right) \cdot \omega\right).\\ &\qquad (\text{Area under apparent viscosity curve from } T_0 \text{ to } T_{max})\end{aligned}$$

$\mathrm{d}t$ Incremental change in time

$\tau_{(t)}$ Shear stress (as a function of time)

ω Rotational speed of spindle (rad·s^{-1})

γ Shear rate (s^{-1})

$\eta_{(t)}$ Viscosity (as a function of time)

R_c Radius of container used for measuring viscosity

2.5 Statistical analysis

Data were analyzed using a factorial randomized block design with method of concentration (control, UF, and CM), protein level (32, 45, and 60 g·kg^{-1}), and rennet inoculation level (132 and 198 μL·kg^{-1}) as the factors, blocked within each replication. Means were compared using Fisher's least significant difference

Table 2 Rennet clotting properties of milks as measured using Formagraph

Parameters	Treatment				
	C	CM1	UF1	CM2	UF2
RCT[a] (min)	33.5 a	22.1 b	23.5 b	18.5 d	21.2 c
k_{20}[b] (min)	44.4 a	8.5 b	8.3 b	4.2 c	4.3 c
a_{40}[c] (mm)	2.8 a	36.5 b	34.5 b	55.3 c	54.2 c

Means in rows with same letters (a–d) do not differ ($P>0.05$). Mean of six replicates

C control (unconcentrated milk (32 g·kg^{-1} protein)), *CM1* condensed milk (45 g·kg^{-1} protein), *UF1* ultrafiltered milk (45 g·kg^{-1} protein), *CM2* condensed milk (60 g·kg^{-1} protein), *UF2* ultrafiltered milk (60 g·kg^{-1} protein)

[a] Rennet clotting time

[b] Time taken for amplitude on Formagraph to reach 20 mm after rennet clotting time

[c] Amplitude on Formagraph after 40 min of rennet addition

procedure. The GLM procedure in SAS Institute Inc. (1990) was used to analyze the data. A 95% level of significance was used for all analyses.

3 Results and discussion

3.1 Formagraph measurements

3.1.1 Rennet clotting time

RCT represents the time for gel formation (McMahon and Brown 1982). RCT ranged from 18.5 to 33.5 min. (Table 2); and was significantly ($P<0.05$) affected by protein level and method of concentration. It decreased ($P<0.05$) as the protein level increased (C vs. CM1 or UF1 vs. CM2 or UF2) but the average RCT of UF treatments were higher ($P<0.05$) than those of CM.

A similar effect of protein levels on RCT of UF milks was observed by Mehaia and El-Khadragy (Mehaia and El-Khadragy 1998). In comparison to UF, CM had a lower ($P<0.05$) RCT. RCT is a result of two overlapping processes: proteolysis and aggregation. This decrease in RCT could be due to increase in rate of aggregation as a result of decrease in volume of the aqueous phase because of higher total solids in CM as compared with its UF counterparts (Mehaia and El-Khadragy 1998). In addition, a lower pH, as a result of increased concentration of Ca, phosphate, and milk-solids-not-fat may have led to an increase in the rate of enzymatic reaction, and hence a lower RCT (Cheryan et al. 1975; Daviau et al. 2000). These results also indicate that heating of milk to a temperature of 60 °C during vacuum condensing, until the desired concentration was obtained, did not prolong RCT.

3.1.2 Rate of curd formation (k_{20})

The parameter, $k_{20,}$ is an indicator of the rate of curd formation after the initial point of gel formation (McMahon and Brown 1982). It is also defined as the point at which the curd is firm enough to be cut during cheese making (McMahon and Brown 1982). The k_{20} values for the milks ranged from 4.2 to 44.4 min (Table 2); and were significantly ($P<0.05$) affected by protein level, i.e., rate of curd formation increased with increasing protein content. Similar results were also observed by Daviau et al. (Daviau et al. 2000), and Kameswaran and Smith (Kameswaran and Smith 1999).

3.1.3 Curd firmness (a_{40})

Curd firmness in a Formagraph is estimated by measuring the width of the graph after 40 min of addition of rennet, and is represented by a_{40}. Values for a_{40} ranged from 2.8 to 55.3 mm (Table 2), and were significantly ($P<0.05$) affected by protein level. Samples with higher protein content exhibited higher a_{40} value. Similar trend was observed by Daviau et al. (Daviau et al. 2000).

Statistical analysis of a_{40} values did not indicate a significant effect of method of concentration on curd firmness. But as apparent from Fig. 1, method of concentration seems to have some effect on the coagulum formation and resistance of coagulum to

break. Firmness for CM curds appears to continue to increase beyond the point of the a_{40} value as compared with their UF counterparts. This was also observed when the CM and UF curds were analyzed subjectively during cheese making (Acharya and Mistry 2004). To compare the rennet coagulum formation/destruction characteristics of concentrated milks, this study was continued using a Brookfield viscometer. Brookfield viscometer provides a complete profile of viscosity until syneresis occurs and the data obtained can be manipulated to understand coagulum characteristics. In this experiment, rennet concentration was also changed to modulate coagulation.

3.2 Brookfield measurements

Despite differences between techniques, the shape of curves (Fig. 1 vs. 2) characterizing rennet coagulation over time are essentially the same. However, in contrast to Formagraph, Brookfield viscometer provides continuous apparent viscosity measurements throughout the experimental run.

3.2.1 Initial viscosity, time of onset, and time to reach maximum viscosity

η_0 was defined as the apparent viscosity after 1 min of commencement of the experiment (Fig. 2). The η_0 values of different treatments ranged from 2.6 to 3.3 mPa·s (Table 3); η_0 was significantly ($P<0.05$) affected by protein level, method of concentration and replications (Table 4). Samples with higher protein exhibited higher η_0 ($P<0.05$). In addition, CM had higher apparent viscosity ($P<0.05$) than UF because of higher total solids content in CM treatments vs. UF treatments.

After addition of rennet to milk, enzymatic action of rennet on caseins continued without any change in apparent viscosity. Thereafter, a sudden onset of increase in apparent viscosity was observed, and this time of onset was defined as T_0 (Fig. 2). T_0 for different treatments ranged from 12 to 22 min (Table 3), and was significantly ($P<0.05$) affected by the rate of rennet inoculation and protein level (Table 4). Treatments with a higher rate of inoculation (i.e., 198 vs. 132 $\mu L \cdot kg^{-1}$) exhibited lower ($P<0.05$) T_0 (Table 3). Also, treatments with higher protein level (C vs. CM1 or UF1 vs. CM2 or UF2) had shorter ($P<0.05$) T_0. Similar results have been reported previously (Culioli and Sherman 1978). Culioli and Sherman (Culioli and Sherman 1978) also observed that the effect of protein on clotting time could be reduced to nil by changing rennet concentration. Considering the high Mean Square value of rennet (Table 4), a similar conclusion can be deduced that the time of onset of increase in apparent viscosity (T_0) can be significantly modified based on rennet concentration.

As the aggregation of casein micelles continues, a significant increase in viscosity was observed and the time when maximum apparent viscosity was reached was defined as T_{max}. T_{max} for different treatments ranged from 17 to 31 min (Table 3), and was significantly ($P<0.05$) affected by protein level, method of concentration, rate of rennet addition, and interaction between protein level and rate of rennet addition (Table 4). In general, an increase in rate of rennet inoculation led to a decrease ($P<0.05$) in T_{max}. However, interaction effect indicates that for the same protein level, with increase in rate of rennet inoculation from 132 to 198 $\mu L \cdot kg^{-1}$ milk, the magnitude of decrease in T_{max} was not similar between different protein treatments ($P<0.05$). The effect of increasing rennet concentration was higher ($P<0.05$) on 45 and 60 $g \cdot kg^{-1}$ protein samples (CM and UF) as compared with the 32 $g \cdot kg^{-1}$ protein samples (C). In addition, CM took longer time

Table 3 Rennet clotting characteristics of milks as measured using Brookfield viscometer

Parameters	Treatment									
	C		CM1		UF1		CM2		UF2	
	132 μL·kg^{-1}	198 μL·kg^{-1}	132 μL·kg^{-1}	198 μL·kg^{-1}	132 μL·kg^{-1}	198 μL·kg^{-1}	132 μL·kg^{-1}	198 μL·kg^{-1}	132 μL·kg^{-1}	198 μL·kg^{-1}
η_0 (mPa·s)	2.65 a	2.64 a	2.91 b, c	2.98 b, e	2.84 c	2.87 c	3.18 f	3.26 f	3.06 d, e	3.08 d
T_0[b] (min)	22.3 a	17.3 d	21.5 a, b	13.8 e, f	21.5 a, b	14.0 e, f	18.8 c, d	14.3 e	19.8 b, c	12.3 f
T_{max} (min)	28.3 a	23.8 c	31.5 b	22.3 c	28.5 a	18.8 d, e	27.8 a	21.0 c, d	28.5 a	17.5 e
η_{max}[d] (mPa·s)	5.12 a	5.03 a	10.03 b	10.63 b	8.27 c	8.65 c	22.93 d	23.50 d	16.30 e	17.68 f
Coagulum strength (N·m)	3.39 a, b	2.72 a	7.03 d, e	6.06 c, d	4.25 b	3.22 a, b	9.22 g	8.25 f, g	7.88 e, f	5.86 c

Means in rows with same letters (a–g) do not differ ($P>0.05$). Mean of four replicates

C control (unconcentrated milk (32 g·kg^{-1} protein)), *CM1* condensed milk (45 g·kg^{-1} protein), *UF1* ultrafiltered milk (45 g·kg^{-1} protein), *CM2* condensed milk (60 g·kg^{-1} protein), *UF2* ultrafiltered milk (60 g·kg^{-1} protein)

[a] Apparent viscosity after 1 min of addition of rennet (measured in mPa·s)

[b] Time at the onset of increase in apparent viscosity

[c] Time when maximum apparent viscosity was reached

[d] Maximum apparent viscosity (measured in mPa·s)

Table 4 Mean squares (MS) and probabilities (in parentheses) of rennet clotting properties as measured by Brookfield viscometer

Source of variation	*df*	η_0[a]	T_0[b]	T_{max}[c]	η_{max}[d]	Coagulum strength
Protein[e]	2	0.701* (<0.01)	33.02* (<0.01)	17.27* (0.02)	756.38* (<0.01)	65.67* (<0.01)
Method[f]	1	0.119* (<0.01)	0.28 (0.69)	42.78* (<0.01)	130.0* (<0.01)	43.73* (<0.01)
Protein × method	1	0.006 (0.23)	0.78 (0.50)	7.03 (0.18)	38.50* (<0.01)	1.77 (0.07)
Rennet[g]	1	0.017 (0.06)	416.03* (<0.01)	6,803.63* (<0.01)	3.25* (0.02)	12.75* (<0.01)
Protein × rennet	2	0.003 (0.51)	5.27 (0.06)	17.97* (0.01)	0.76 (0.24)	0.52 (0.37)
Method × rennet	1	0.005 (0.27)	3.78 (0.14)	11.28 (0.09)	0.16 (0.57)	0.61 (0.28)
Protein × method × rennet	1	0.007 (0.69)	5.28 (0.09)	7.03 (0.18)	0.53 (0.32)	0.49 (0.33)
Replication	3	0.018* (0.02)	1.35 (0.50)	1.29 (0.78)	1.53* (0.05)	0.26 (0.67)
Error	27	0.004	1.67	3.63	0.50	0.50

*$P<0.05$, significant

[a] Apparent viscosity after 1 min of addition of rennet (measured in mPa·s)

[b] Time at the onset of increase in apparent viscosity

[c] Time when maximum apparent viscosity was reached

[d] Maximum apparent viscosity (measured in mPa·s)

[e] Level of protein: 32 g·kg^{-1} (C), 45 g·kg^{-1} (CM1 and UF1), and 60 g·kg^{-1} (CM2 and UF2)

[f] Method of concentration: no concentration (C), ultrafiltration (UF1 and UF2), and condensing (CM1 and CM2)

[g] Inoculation rate of rennet (132 or 198 μL·kg^{-1} of milk)

($P<0.05$) to reach maximum viscosity as compared with UF or C. The high values of Mean Square of rennet (Table 4) indicate that the T_{max} can also be significantly modified using rate of rennet inoculation.

The η_{max} for different treatments, at their respective T_{max}, ranged from 5 to 23 mPa·s (Table 3). The η_{max} was significantly ($P<0.05$) affected by protein level, method of concentration, rate of rennet inoculation, replications, and interaction effect of protein level and method of concentration (Table 4). Treatments with higher rate of rennet inoculation, in general, had higher ($P<0.05$) η_{max}. Similar effect of increase in curd firmness with increasing rennet concentration was also observed by Okigbo et al. (Okigbo et al. 1985). The interaction effect of method of concentration and protein level indicates that for 45 g·kg^{-1} protein treatments (CM1 and UF1), average η_{max} was similar irrespective of method of concentration (CM or UF) (Table 3); however, for 60 g·kg^{-1} protein treatments, η_{max} was significantly higher ($P<0.05$) for CM than UF. Differences in η_{max} for CM2 vs. UF2 are measurable in Brookfield viscometer, and are in agreement with what was observed subjectively during cheese manufacturing.

In contrast to T_0 and T_{max}, mean square values for rennet are not high for η_{max}, indicating a minor influence of rate of rennet inoculation on η_{max} as compared with other

factors, such as protein level or method of concentration (Table 4). This was also observed by Culioli and Sherman (Culioli and Sherman 1978), who reported that rennet concentration influenced the clotting time, while the retentates protein content influenced the ultimate value of gel firmness.

3.2.2 Gelation process during viscometry and coagulum strength

Observations by electron microscopy (Green et al. 1978) have revealed that the continuing gelation process involves specific interactions between the chain of casein micelles that cause them to align and form strands. This is observed as a rapid, accelerated, increase in viscosity or rigidity of the milk gel, measured by non-destructive or destructive methods. However, during viscosity measurements using a rotating cylinder in Brookfield viscometer, the continuously forming new inter-micellar bridges are partly destroyed. During the initial phase of the milk clotting, the rate of formation of new bridges is higher than the rate of disruption, which results in increasing viscosity. When the rate of formation slows down, and becomes lower than the rate of disintegration, the viscosity level reaches a maximum (η_{max}) and diminishes. The total energy required to disintegrate the coagulum can be calculated (see "Materials and methods") and can be used as an indicator of coagulum strength. Because of the continuous disruption of network during the experiment, these values do not represent true coagulum strength; however, can be used to identify relative differences between treatments.

The coagulum strength for different treatments varied from 2.7 to 9.2 N·m (Table 3); and was significantly ($P<0.05$) affected by protein level, method of concentration, and rate of rennet inoculation (Table 4). Samples with higher protein concentration, in general had higher ($P<0.05$) coagulum strength. In addition, CM treatments exhibited higher ($P<0.05$) coagulum strength as compared with their UF counterparts. Coagulum strength was observed to be altered by varying the rate of rennet inoculation. A higher rate of rennet inoculation exhibited lower ($P<0.05$) coagulum strength. At a high level of rennet inoculation (198 $\mu L \cdot kg^{-1}$), a more viscous gel is formed (i.e., high η_{max}) in a shorter time (i.e., low T_{max}). However, the gel formed is more susceptible to breakage (i.e., low coagulum strength). In contrast, at a low level of rennet inoculation (132 $\mu L \cdot kg^{-1}$), a less viscous gel is formed (i.e., low η_{max}) in a longer time (i.e., high T_{max}); and the gel formed is more resilient to breakage (i.e., high coagulum strength).

4 Conclusions

Our results indicate that there are significant differences in rennet curd formation characteristics of milk with different protein levels. In addition UF and CM curds and their susceptibility to damage differ from each other. Coagulation of concentrated milk blends used for cheese making should ideally follow a path where sufficient soluble casein can be incorporated into the coagulum, without excessive increase in curd firmness. This can be achieved, to some extent, by altering the amount of rennet used during cheese making. A decrease in rate of rennet addition may increase the RCT to be acceptable for practical applications; however, this may increase the coagulum strength, and alter the final curd characteristics. A further analysis on aggregation and gelation kinetics vs. rheology can help understand the coagulation mechanism of concentrated milk (CM or UF) and ways to modulate the gel characteristics. Another avenue

to study may be the syneresis behavior of these gels. As apparent from Fig. 1, although the UF gels form at the same rate as CM, at a later time, there is a decrease in movement of the pendulum that is indicative of increased syneresis of the gel. So for a cheese-making application, a faster rate of contraction of the gel and syneresis after the cheese curd has been cut would be advantageous.

Acknowledgments The authors gratefully acknowledge the assistance of David Moonay, from Brookfield Engineering Laboratories, Inc., for consultation in data analysis and Midwest Dairy Foods Research Center for providing funds.

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