

Full Length Research Paper

Clotting of cow (*Bos taurus*) and goat milk (*Capra hircus*) using calve and kid rennets

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The ease to locally produce kid rennet contrary to that of calve has led us to compare the proteolytic and clotting activities of these two rennets depending on their action on goat (*Capra hircus*) milk and cow (*Bos taurus*) milk. The proteolysis was measured by determining the increase of non-protein nitrogen according to the kjeldalh method and milk coagulation was monitored with the aid of a formagraph. Each rennet hydrolyses more completely the casein of its own specie. With cow milk, the coagulation, same as the curd firmness rate of the gels obtained were practically similar independent on the origin of rennet. With goat milk, the two rennet significantly influenced the coagulation and the curd firmness rate of the gel. It was concluded that contrary to goat milk, cow milk can be coagulated indifferently by calve or kid rennet. The hardening of its gel is independent of the type of rennet.

Key words: Coagulum, cow, goat, milk clotting, proteolysis, rennet.

INTRODUCTION

Between 1996 and 2002, the quantity of Cameroons imported milk and milk products increases from 3×10^8 to more than 10×10^8 tons (Figure 1) representing US \$ 3×10^6 to 8×10^6 . Meanwhile, in 2000, Cameroon was having more than 3.2 millions bovines, more than 2.2 ovines and more than 2.5 caprines (Ministry of Economy and Finance, 2001). Much effort has been put into animal rearing and local milk production. This has led to improvements in the production of local milk products. Due to increasing demands for the consumption of dairy products, industrialists have shifted not only the production of fermented milk (Libouga et al., 2005) but also the production of cheese. The main problem encountered by local cheese manufacturers is the absence of rennet in the local markets. The slaughtering of caves of the species zebu (*Bos indicus*) is forbidden by the law (decree 62/22/COR of 9 March 1962). On the contrary, the slaughtering of kids (*Capra hircus*) is not prohibited by the law. It is therefore, possible to produce kid rennet

at the spot which is currently used in cheese manufacture (Verdalet-Guzman, 1992; Calvo and Fontecha, 2004). Kid chymosin has been purified; its molecular weight is 36 kDa; it is stable at 55°C and has a maximum activity at 37°C. Its clotting activity decreases steadily with increasing pH and has a maximum activity at pH 5.5. The proteolytic activity increases with incubation time at 37°C. The kid chymosin is more stable than that of calve (Kumar et al., 2006).

The question arises whether the proteolytic and clotting properties of kid rennet are similar to those of calve rennet when they are allowed to react one after another on goat and cow milk. Many authors have been interested in kid rennet (Nelson et al., 1977; Hyslop et al., 1979; Camacho et al., 1991; Calvo and Fontecha 2004; Vega Hernandez et al., 2004) and calve rennet. (Somers et al., 2003; Ardisson-Korat and Rizvi, 2004; Zhong et al., 2004; Dwyer et al., 2005; Leiber et al., 2005; Madadlou et al., 2005). The proteolytic activities of calve rennet on cow casein (Ernstrom and Wong, 1983) as well as that of goat rennet on goat casein are specific reactions (Awad et al., 1999). The coagulation of milk is a process that takes place in two steps (Hyslop et al., 1979): the first reaction during which casein κ is hydrolysed and a se-

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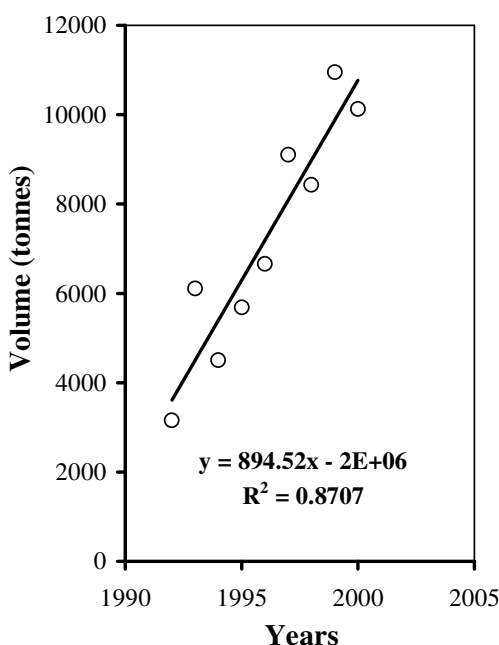


Figure 1. Evolution of milk and milk products between 1992 and 2003. (Ministry of Economy and Finances - Cameroon, 2005).

cond reticular step during which the micelles of para κ casein organises themselves to form a three dimensional net work; the calcium ions form linkages between para casein κ molecules. Alexander and Dalgleish (2004) followed this coagulation using spectroscopic methods and this modelling of goat milk clotting was put into existence (Castillo et al., 2003). The aim of this study is to compare milk clotting and proteolytic activities of kid and calve rennets when acting on goat and on cow milk.

MATERIALS AND METHODS

Milk

Cow (*Bos taurus*) and goat (*Capra hircus*) milk are of great mixture. They were subjected to thermal treatment (30 – 35°C for 10 min) and are skimmed. They were further conserved by the addition of toluene (0.5 mL×L⁻¹) and conserved in a refrigerator. They are used within the first 3 – 4 days. To the fresh milk 0 – 30 mM CaCl₂.2H₂O was added. The mixture was allowed to stand 4 h for equilibrium; after the first 2 h, the pH was adjusted to 6.7 by addition of diluted NaOH and the new pH is verified; this pH is eventually brought to 6.7. Other milk samples were added under strong agitation of NaOH (10 N) or concentrated lactic acid. These samples were allowed to stand 4 h for equilibrium and their pH after 3.5 further hours was noted.

Caseins

Cow milk as well as goat milk were skimmed and diluted 4 times with distilled water and 0.5 M acetic acid was added until pH ≈ 4.6.

The precipitate was collected by centrifugation, washed with distilled water and dissolved again in 0.5 M NaOH (pH ≈ 7). The caseinates obtained were conserved under freeze drying form (Johnson, 1983).

Rennet solutions

Calve rennet is a commercial rennet (Boll Establishments, Arpajon, France); 99.5% of the activity is due to chymosin. Its *force* is 1/10,000. The kid rennet is prepared as follow: abomasums of slaughtered kid were washed and dried in a well ventilated oven (40°C, 12 h), cut into small pieces and then 52.5 g were introduced in 700 ml 7.5% NaCl, 3% H₃BO₃ according to Zalaza and Reinheimer (1980) at 4°C for 3 days. The liquid discarded from tissues is added to 0.2% KAl(SO₄)₂.12H₂O concentrated solution. The pH drops to approximately pH 4 and it was brought to pH 2 for about an hour. After which, it is taken to pH 5.5 by addition of Na₂HPO₄ (Riedel-de Haën) according to Valles and Furret (1977).

Chromatography

A Sepharose gel containing quinone is prepared as follows: a mixture of 200 mL of Sepharose 4B (Pharmacia Fine Chemicals), 120 mL of 0.10 M benzoquinone (Merck), and 400 mL 0.05 M, pH 8.0 phosphate buffer is agitated gently in obscurity for 24 h. The gel obtained is washed with 500 mL 0.05 M, pH 8.0 phosphate buffer and it was subjected to a slow agitation in the presence of 300 mL 0.1 M, pH 8.0 according to Gregorio Di et al. (1979a). This gel is conserved at 4°C in presence of NaN₃ and is used within the first 30 days. The gel of 30 cm length was put in the column (42 × 1.1 cm); the sample (2 mL) was eluted with 0.1 M, pH 6.7 phosphate buffer with a flow rate of 9.5 mL/h. Collection of fractions was made using Microcol TDC 80 (Gilson). Fractions of 5 mL were collected. The optical density was obtained by passing different fractions through a spectrophotometer at 280 nm using a 1.0 cm length curvet.

Measuring of milk clotting time

The determination of milk clotting time was done according to Berridge (1952) and Collin et al. (1977). Reconstituted milk (Berridge substrate) was obtained by dissolving 12 g of low heat powder milk (INRA, Poligny, France) in 100 mL of 0.01 M CaCl₂.2H₂O. To 10 mL of this reconstituted milk in a test tube were added 0.5 mL enzymatic solution and put in a water bath at 30°C. The test tube was submitted to a slight rotation until a layer of film of milk appears inside of the wall of the test tube. The time obtained is the mean of three trials.

Quantity of chymosin

The milk clotting activity is expressed in rennet unit (RU) according to the following relation:

$$RU = (10 \times V) / (T \times v)$$

V is the milk volume (mL); v is the chymosin, it is calculated according enzymatic solution (mL), T is the milk clotting time in seconds. The quantity of chymosin is calculated according to Gregorio Di et al. (1979b). It is substrate from the total milk clotting activity (RU_T) of the sample the milk clotting activity of the first peak (RU_F). The quantity of chymosin is obtained by the following formula:

$$\% \text{ of chymosine} = [(RU_T) - (RU_F)] \times 100 / (RU_T)$$

Determination of the rennet *force*

The rennet *force* was calculated using the following formula:

$$F_x \times C_x \times t_x = F_s \times C_s \times t_s$$

Where F_x , C_x and t_x are respectively the force, the concentration, and the milk clotting time of the reference rennet and F_s , C_s and t_s are that of the unknown rennet.

Physicochemical parameters determinations

The pH is determined using a glass electrodes pH meter. The titrable acidity is measured using N/9 NaOH. To 10 mL of milk, containing some drops of phenolphthalein were added N/9 NaOH until the appearance of rose colour. The number of mL of N/9 mL NaOH corresponds to the quantity of acidity in dornic degree ($^{\circ}D$) (Recueil des Normes Françaises, 1980). The nitrogen content was determined using the kjeldahl method (Recueil des Normes Françaises, 1980), using a selenium catalyst. Determination of calcium ions was done with flame atomic absorption (Z-8 100 Polarized Zeeman de Hitachi) apparatus. The supplying circuit is rinsed with Millipore water and the standard solutions are injected one after another. The sample was injected after cleaning of the supplying circuit with Millipore water.

Proteolytic activity determination

The 2% (w/v) cow or goat casein solutions are obtained by dissolving freeze dried casein in 25 mM, pH 6.7. Kid rennet were concentrated 3.5 times by filtration using Amicon cell with a membrane of 10,000 cut-off. The dilute calve rennet and the concentrated kid rennet had the same *force* using Berridge method (1952). The mixture rennet/casein solution (1%) (v/v) was incubated in a water bath at 35°C and periodically an aliquot of the mixture was taken and mixed with trichloroacetic acid at the final concentration of 12% according to Rowland (1938) and Garnier (1957). An aliquot of the liquid filtrate (0.2 μ porosity Millipore membrane) of this solution (NPN: none protein nitrogen) as well as a sample of the mixture rennet and casein solution (TN: total nitrogen) were submitted to kjeldahl analysis; at different times, variations of none protein nitrogen (% Δ NPN / NT) were expressed as follow:

$$\% \Delta \text{ NPN} / \text{TN} = 100 \times (\text{NPN}_t - \text{NPN}_0) / \text{TN}$$

Where NPN_0 and NPN_t are respectively the values of none protein nitrogen at the beginning and the time "t".

Formagraph measurements

The formagraph is formed by a pendulum plunged into a bowl containing the mixture casein : rennet (Libouga et al., 2002). The clotting of milk is plotted on a photo sensible paper moving at the rate of 2 mm/min. Two main parameters "r" and "k₂₀" are defined on the formagramm. "r" measures the time between the time when renneting and the instant where the separation between the two branches of the formagramm reaches 1 mm while k₂₀ measures the time between the moment of renneting and the instant where the two branches space reaches 20 mm. For "r" or "k₂₀". The value ob-

tained when adding 0 mM CaCl₂.2H₂O is considered to be 100 and all the other values are expressed proportionally (Proportional numbers).

Plotted formagramm

Formagramm are scanned and plotted using ungraph software (Biosoft United Kingdom).

Statistical analysis

Variance analysis (ANOVA) are used to compare average using SAS (User's Guide, 1985) software.

Mathematical model for none protein nitrogen

$$Y_{ij} = \mu + C_i + T_j + (C^*T)_{ij} + e_{ij}$$

Where: Y_{ij} is the percentage of none protein nitrogen for the i^{th} hydrolysis of casein by rennet hydrolysis after the j^{th} time, μ is the general average, C_i is the effect of the casein, i^{th} rennet hydrolysis with $i = (\text{CCCR}, \text{CCKR}, \text{GCCR}, \text{GCKR})$, T_j is the effect of the j^{th} time with $j = (0, 1, 2, 4, 6, 8, 10, 12, 15, 20, 25, 30, 45, 60, 90 \text{ min})$, $(C^*T)_{ij}$ is the effect of interaction between the i^{th} hydrolysis and the j^{th} time, e_{ij} is the error effect of the percentage of none protein nitrogen on the i^{th} hydrolysis after the j^{th} time.

Mathematical model for coagulation

$$Y_{ab} = \mu + L_a + T_b + (L^*T)_{ab} + e_{ab}$$

Y_{ab} is the milk clotting time for the a^{th} action of rennet on milk with the b^{th} quantity of CaCl₂.2H₂O. μ is the general average. L_a is the effect of a^{th} action of rennet on milk with $a = (\text{CMCR}, \text{CMKR}, \text{GMCR}, \text{GMKR})$. T_b is the effect of the b^{th} quantity of CaCl₂.2H₂O with $b = (0, 5, 10, 15, 20, 30 \text{ mM})$. $(L^*T)_{ab}$ is the effect of the interaction between the a^{th} action of rennet on milk and the b^{th} quantity of CaCl₂.2H₂O. e_{ab} is the effect on the milk clotting time of the a^{th} action of rennet on milk with the b^{th} quantity of CaCl₂.2H₂O.

Mathematical model for curd firmness

$$Y_{kl} = \mu + P_k + T_l + (P^*T)_{kl} + e_{kl}$$

Where: Y_{kl} is the time of firmness of the curd for the k^{th} action of rennet on milk with the l^{th} quantity of CaCl₂.2H₂O. μ is the general average. P_k is the effect of the k^{th} action of rennet on milk with $k = (\text{CMCR}, \text{CMKR}, \text{GMCR}, \text{GMKR})$. T_l is the effect of the l^{th} quantity of CaCl₂.2H₂O on milk with $l = (0, 5, 10, 15, 20, 30 \text{ mM})$. $(P^*T)_{kl}$ is the effect of the interaction between the k^{th} action of rennet on the milk and the l^{th} quantity of CaCl₂.2H₂O. e_{kl} is the effect of curd firmness time of the error on the k^{th} action of rennet on milk with the l^{th} quantity of CaCl₂.2H₂O.

RESULTS AND DISCUSSION

Table 1 indicates the physicochemical characteristics of cow and goat milks: pH, acidity, total solid, total nitrogen, none protein nitrogen and calcium. The physicochemical

Table 1. Physicochemical characteristics of cow and goat milks.

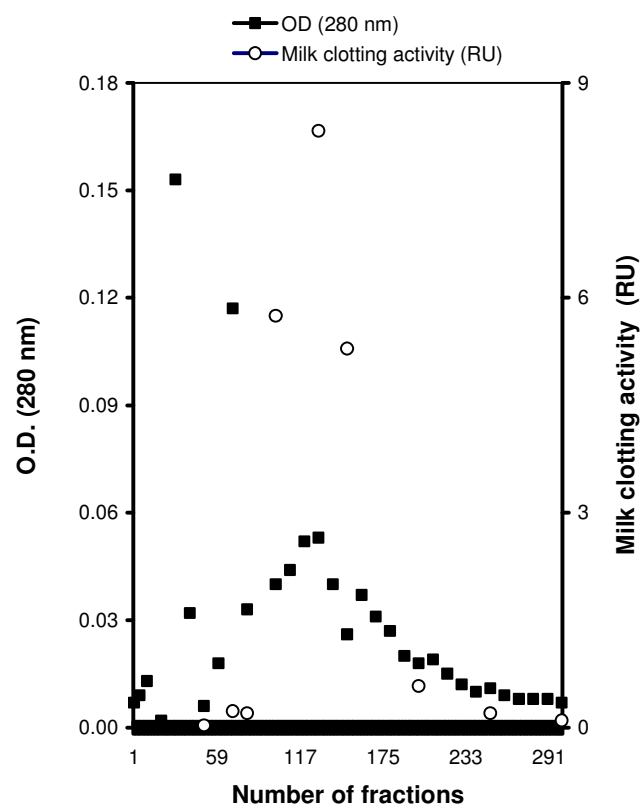
Milk	pH	Acidity ($^{\circ}\text{D}$)	TN ($\text{g} \times \text{L}^{-1}$)	NPN ($\text{g} \times \text{L}^{-1}$)	Total calcium ($\text{g} \times \text{L}^{-1}$)
Goat milk	6.7- 7.0	15 – 19	4.20	0.49	1.12 – 1.43
Cow milk	6.7-6.9	17 – 20	4.62	0.31	1.07

$^{\circ}\text{D}$ = Dornic degree; NPN = none protein nitrogen; TN = total nitrogen.

characteristics of cow and goat milks: pH, acidity, total solid, total nitrogen, none protein nitrogen, calcium. Fresh goat and cow milk have almost identical pH and acidity values. Goat and cow milk protein are 24 and 27 $\text{g} \times \text{L}^{-1}$ respectively. These values are smaller than that usually published (Johnson, 1983). Some authors indicated that chemistry compositions of goat and cow milk are different (Verdalet-Guzman, 1992). Calcium content in goat and cow milk is the same as that found in literature (Johnson, 1983). Figure 2 is a chromatogram obtained by performing the bio specific chromatography of kid rennet.

Chymosin content in kid rennet is closed to 95% of activity. A 1% calve rennet solution corresponds to 3.5% kid rennet solution. Table 2 shows that the schemes of the proteolysis of goat casein and that of a cow under the actions of either kid or calve rennet are similar: kid rennet hydrolyses cow and goat casein just as calve rennet. The percentage of proteolysis of goat casein by the kid rennet is significantly ($p < 0.05$) different from that due to calve rennet and vice versa. The proteolytic activity of kid rennet is well-known (Calvo and Fontecha, 2004). Each rennet proteolyzes its own casein better than that of other species.

Casein proteolysis either with kid or with calve rennet on casein are specific reaction and do not change cheese yield. Camacho et al. (1991) found the same conclusion when making cheese with goat milk using either kid or calve rennet. Figure 3 illustrates the formagramm obtained by allowing kid rennet to react with goat milk at pH 7.0. Figure 4 and Table 3 show the variation of milk clotting time of goat and cow milk under actions of kid, and calve rennet as a function of the pH of milks and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ added in milk. Figure 5 and Table 4 illustrate the evolution of goat and cow curd firmness under actions of kid and calve rennet as function of milk added $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$. Between pH 5 and 7.5, there is no significant difference between curd firmness time of goat and cow milks under calve and kid rennets. With milk added $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, the cow milk firmness time does not vary with type of rennet contrary to goat milk. Between pH 5 and 8, there is no significant difference ($p < 0.05$) between milk clotting time of goat and cow milk under the actions of kid and calve rennet. In the presence of milk added $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0 – 20 mM), the milk clotting time varies significantly ($p < 0.05$) according to goat or cow milk. Between 20 – 30 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ added milk, Ca^{2+} ions delays milk clotting time. During milk pH modification, the

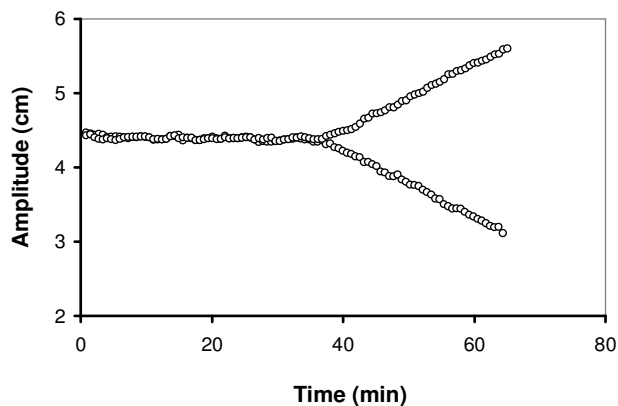
**Figure 2.** Chromatogram of a biospecific chromatography of kid rennet.

main phenomena should be casein demineralisation followed by its hydrolysing. The increasing of calcium concentration in goat or cow milk results in milk clotting time decreasing when using either kid or calve rennet. During milk clotting, calcium ions makes bridges between micelles of para casein indicating that, the more there are calcium ions (0 – 20 mM) in milk, the more there will be linkage and the faster will be milk clotting. At concentrations higher than 20 mM added $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, Ca^{2+} inhibits milk clotting network. When using kid or calve rennet, milk clotting time decreases faster when using cow milk than goat milk. Goat milk micelles are bigger than cow milk micelles. Milk clotting time due to chymosine depends on micelles size (Ekstrand et al., 1980; Omar, 1985; O'Connel and Fox, 2000); it decreases while micelles size increases. For the same volume, there are

Table 2. Changes in non-protein nitrogen ($\Delta\%$ NPN/NT) with incubation time.

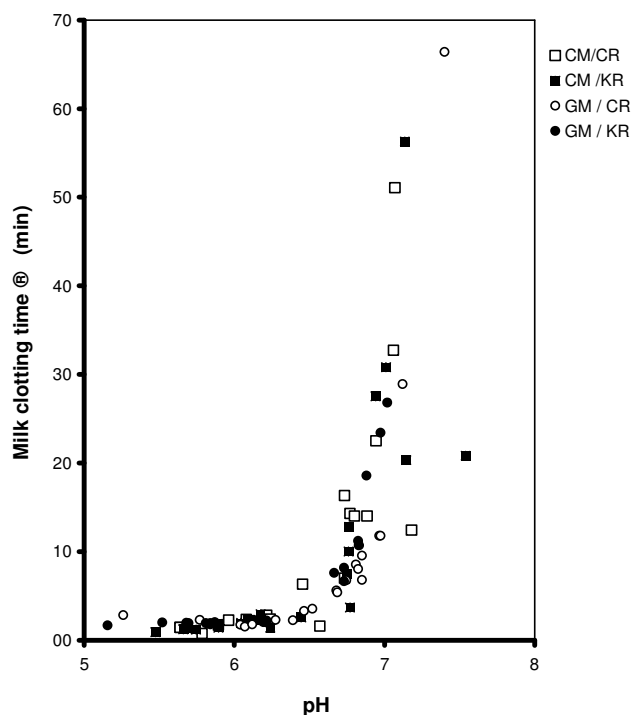
Time (min)	$\Delta\%$ NPN/NT			
	CC/CR	CC/KR	GC/CR	GC/KR
0	0 ± 0.017 ^{a,1}	0 ± 0.017 ^{a,1}	0 ± 0.017 ^{a,1}	0 ± 0.017 ^{a,1}
1	0.56 ± 0.017 ^{a,2}	0.62 ± 0.017 ^{b,2}	0.15 ± 0.017 ^{c,2}	0.30 ± 0.017 ^{d,2}
2	0.63 ± 0.017 ^{a,3}	0.67 ± 0.017 ^{b,3}	0.34 ± 0.017 ^{c,3}	0.60 ± 0.017 ^{a,d,3}
4	0.74 ± 0.017 ^{a,4}	0.75 ± 0.017 ^{a,4}	0.56 ± 0.017 ^{b,4}	0.76 ± 0.017 ^{a,4}
6	0.99 ± 0.017 ^{a,5}	0.91 ± 0.017 ^{b,5}	0.72 ± 0.017 ^{c,5}	1.14 ± 0.017 ^{d,5}
8	1.21 ± 0.017 ^{a,6}	1.11 ± 0.017 ^{b,6}	0.80 ± 0.017 ^{c,6}	0.99 ± 0.017 ^{d,6}
10	1.30 ± 0.017 ^{a,7}	1.15 ± 0.017 ^{b,6,7}	0.78 ± 0.017 ^{c,6,7}	1.03 ± 0.017 ^{d,6,7}
12	1.36 ± 0.017 ^{a,8}	1.23 ± 0.017 ^{b,8}	0.87 ± 0.017 ^{c,8}	0.98 ± 0.017 ^{d,6,7,8}
15	1.39 ± 0.017 ^{a,8,9}	1.16 ± 0.017 ^{b,7,9}	0.95 ± 0.017 ^{c,9}	1.13 ± 0.017 ^{d,b,5,9}
20	1.43 ± 0.017 ^{a,10}	1.25 ± 0.017 ^{b,8,10}	0.91 ± 0.017 ^{c,8,9,10}	1.02 ± 0.017 ^{d,6,7,8,10}
25	1.32 ± 0.017 ^{a,7,8,11}	1.23 ± 0.017 ^{b,8,10,11}	1.24 ± 0.017 ^{b,c,11}	1.04 ± 0.017 ^{d,6,7,10,11}
30	1.41 ± 0.017 ^{a,9,10,12}	1.38 ± 0.017 ^{a,b,12}	1.10 ± 0.017 ^{c,12}	1.11 ± 0.017 ^{c,d,5,9,12}
45	1.59 ± 0.017 ^{a,13}	1.30 ± 0.017 ^{b,13}	0.98 ± 0.017 ^{c,9,13}	1.19 ± 0.017 ^{d,13}
60	1.52 ± 0.017 ^{a,15}	1.40 ± 0.017 ^{b,12,14}	0.99 ± 0.017 ^{c,9,13,14}	1.17 ± 0.017 ^{d,5,9,13,14}
90	1.71 ± 0.017 ^{a,15}	1.38 ± 0.017 ^{b,12,14,15}	1.04 ± 0.017 ^{c,15}	1.22 ± 0.017 ^{d,13,15}

Values in the same line having the same letter (a, b, c, d ...) in exponent are not significantly different at the threshold of 5%. The values in the same column having the same number (1, 2, 3, 4 ...) in exponent are not significant.

**Figure 3.** Formagramm obtained by kid rennet on cow milk (raw picture was scanned and drawn again using Ungraph software).

more bridges between para casein micelle in cow milk than in goat milk.

Para casein micelle network is formed more rapidly with cow milk than with goat milk. The proteolysis of cow casein by kid rennet is not complete compare to calve rennet but since cow milk micelles are relatively small, cow milk coagulation is not limited by the number of para casein micelles capable to form bridge with calcium ions. It is the same conclusion when considering the firmness of the gel obtained with cow milk reacting with kid and calve rennets. Different results are obtained with goat milk casein micelles which are bigger than cow milk casein micelles (Verdalet-Guzman, 1992). This can be

**Figure 4.** Variation of milk clotting time (r) of goat milk (GM) and cow milk (CM) pH under actions of kid (KR) and calve rennets (CR).

explained by the fact that, proteolysis of goat casein by calve rennet is less complete than with kid rennet. The coagulation of goat milk is limited by the quantity of para

Table 3. Variation of milk clotting time with quantity of CaCl₂.2H₂O added.

CaCl ₂ .H ₂ O (mM)	Milk clotting time (proportional numbers)			
	CC/CR	CC/KR	GC/CR	GC/KR
0	100 ± 1.46 ^{a.1}	100 ± 1.46 ^{a.1}	100 ± 1.46 ^{a.1}	100 ± 1.46 ^{a.1}
5	52.66 ± 1.46 ^{a.2}	54.27 ± 1.46 ^{a.b.2}	72.02 ± 1.46 ^{c.2}	78.25 ± 1.46 ^{d.2}
10	39.00 ± 1.46 ^{a.3}	40.47 ± 1.46 ^{a.b.3}	63.47 ± 1.46 ^{c.3}	70.22 ± 1.46 ^{d.3}
15	31.00 ± 1.46 ^{a.4}	33.36 ± 1.46 ^{a.b.4}	61.47 ± 1.46 ^{c.3.4}	64.96 ± 1.46 ^{c.d.4}
20	28.00 ± 1.46 ^{a.4.5}	28.81 ± 1.46 ^{a.b.5}	59.94 ± 1.46 ^{c.3.4.5}	65.93 ± 1.46 ^{d.4.5}
30	21.66 ± 1.46 ^{a.6}	29.23 ± 1.46 ^{b.5.6}	59.79 ± 1.46 ^{c.3.4.5.6}	77.70 ± 1.46 ^{d.2.6}

Values in the same line having the same letter (a, b, c, d ...) in exponent are not significantly different at the threshold of 5%. The values in the same column having the same number (1, 2, 3, 4 ...) in exponent are not significantly different at the threshold of 5%.

Table 4. Variation curd firmness time with quantity of CaCl₂.2H₂O added.

CaCl ₂ .H ₂ O (mM)	Curd firmness (proportional numbers)			
	CC/CR	CC/KR	GC/CR	GC/KR
0	100 ± 0.9 ^{a.1}	100 ± 0.9 ^{a.1}	100 ± 0.9 ^{a.1}	100 ± 0.9 ^{a.1}
5	43.30 ± 0.9 ^{a.2}	46.45 ± 0.9 ^{a.b.2}	82.0 ± 0.9 ^{c.2}	54.08 ± 0.9 ^{d.2}
10	35.27 ± 0.9 ^{a.3}	38.49 ± 0.9 ^{b.3}	63.80 ± 0.9 ^{c.3}	50.99 ± 0.9 ^{d.3}
15	31.25 ± 0.9 ^{a.4}	32.87 ± 0.9 ^{a.b.4}	70.0 ± 0.9 ^{c.4}	50.95 ± 0.9 ^{d.3.4}
20	29.46 ± 0.9 ^{a.4.5}	34.60 ± 0.9 ^{b.4.5}	91.20 ± 0.9 ^{c.5}	54.95 ± 0.9 ^{d.2.5}
30	37.95 ± 0.9 ^{a.6}	38.67 ± 0.9 ^{b.a.b.3.6}	136.20 ± 0.9 ^{c.6}	82.80 ± 0.9 ^{d.6}

Values in the same line having the same letter (a, b, c, d ...) in exponent are not significantly different at the threshold of 5%. The values in the same column having the same number (1, 2, 3, 4 ...) in exponent are not significantly different at the threshold of 5%.

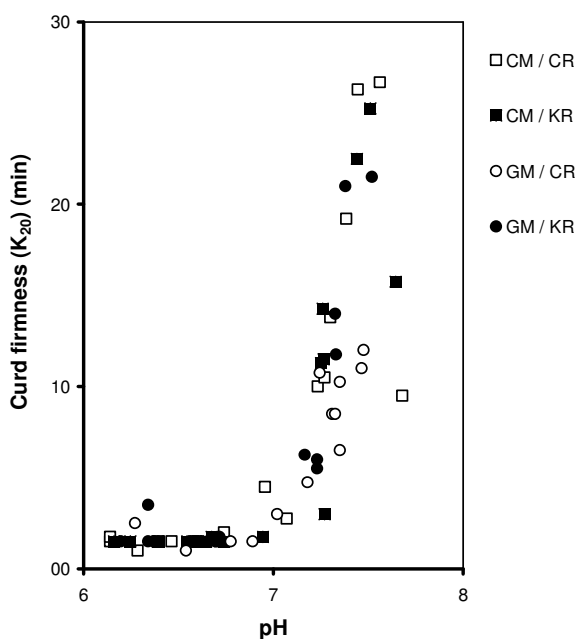


Figure 5. Evolution of curd firmness time (k_{20}) of goat milk (GM) and cow milk (CM) pH obtained with actions of kid rennet (KR) and calve rennet (CR).

casein micelles between which bridges are formed through calcium ions. Goat milk clotting, as also the firmness of its gel are made faster with kid rennet than with calve rennet. Between 20 and 30 mM added CaCl₂.2H₂O, Ca²⁺ acts as milk clotting inhibitor. Kid and calve rennet can be used to clot cow milk. With cow milk, the curd formation and its firmness are similar when using kid rennet or calve rennet. Proteolysis of cow casein is not as total as that of calve. On the contrary, when using goat milk, kid rennet gives best milk clotting time than that of calve and its gel is faster firm up.

Conclusion

The actions of calve and kid rennet are different depending on the milk on which they react. With cow milk, the coagulation as well as the curd firmness rate of the gels obtained were practically similar independent on the origin of rennet. With goat milk, the two rennets significantly influence the coagulation and the curd firmness rate of the gel. Contrary to goat milk, cow milk can be coagulated indifferently by calve or kid rennet. The hardening of its gel is independent of the type of rennet.

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REFERENCES

- Alexander M, Dalgleish DG (2004). Application of transmission diffusing wave spectroscopy to the study of gelation of milk by acidification and rennet. *Colloids Surf. B. Biointerfaces* 38: 83-90.
- Ardisson-Korat AV, Rizvi SS (2004). Vatless manufacturing of low-moisture part skim mozzarella cheese from highly concentrated skim milk microfiltration retentates. *J. Dairy Sci.* 87: 3601-3613.
- Awad S, Luthi-Peng QQ, Puhan Z (1999). Proteolytic activities of suparen and rennilase on buffalo, cow and goat whole casein and beta-casein. *J. Agric. Food Chem.* 47: 3632-3639.
- Berridge NJ (1952). An improved method of observing the clotting of milk containing rennin. *J. Dairy Res.* 9: 328-329.
- Calvo MV, Fontecha J (2004). Purification and characterization of a pregastric esterase from a hygienized kid rennet paste. *J. Dairy Sci.* 87: 1132-1142.
- Camacho L, Sierra C, Jarpa J, Retamal E (1991). The use of appropriate technologies to improve the sanitary quality and the yield of goat cheeses in little farms. *Arch. Latinoam. Nutr.* 41: 79-91.
- Castillo MZ, Payne FA, Hicks CL, Laencina JS, Lopez MB (2003). Modelling casein aggregation and curd firming in goats' milk from backscatter of infrared light. *J. Dairy Res.* 70: 335-348.
- Collin JC, Grappin R, Legraet Y (1977). Étude de la mesure selon Berridge du temps de coagulation du lait additionné d'une solution enzymatique. *Rév. Lait. Franç.* 355: 391-394.
- Dwyer C, Donnelly L, Bucklin V (2005). Ultrasonic analysis of rennet-induced pre-gelation and gelation processes in milk. *J. Dairy Res.* 72: 303-310.
- Ekstrand B, Larsson-Raznikiewicz M, Perlmann C (1980). Casein micelle size and composition related to the enzymatic coagulation process. *Biochem. Biophys. Acta.* 930: 361-366.
- Ernstrom CA, Wong NP (1983). Milk clotting enzymes and cheese chemistry milk *in*: Webb BH, Johnson AH, Alford JA. *Fundamentals of Dairy Chemistry* edited by The Avi Publishing Company, Inc. Westport, Connecticut; USA.
- Garnier J (1957). Étude de la libération d'azote non protéique dans les laits de différentes espèces animales. *Ann. Inst. Nat. Rech. Agron. Ser. E (Ann. Tech. Agric.)* 3: 245-256.
- Gregorio FDI, Sisto R, Morisi F (1979a). Biospecific chromatography of chymosin on quinonated sepharose and its application to enzyme content determination in rennets. *J. Dairy Res.* 46: 673-680.
- Gregorio FDI, Sisto R, Morisi F (1979b). Metodo di determinazione selettiva della chimasi nei cagli. *Scienza e Tecnica Lattiero casearia* 3: 122-128.
- Hyslop DB, Richardson T, Ryan DS (1979). Kinetics of pepsin-initiated coagulation of kappa-casein. *Biochem. Biophys. Acta* 566: 390-396.
- Johnson AH (1983). The composition of milk *in*: Webb BH, Johnson AH, Alford JA. *Fundamentals of Dairy Chemistry* edited by The Avi Publishing Company, Inc. Westport, Connecticut ; USA.
- Kumar A, Sharma J, Mohanty AK, Grover S, Batish VK (2006). Purification and characterization of milk clotting enzyme from goat (*Capra hircus*). *Comp. Biochem. Physiol. B. Biochem. Mol. Biol.* 145(1): 108-113.
- Leiber F, Nigg D, Kunz C, Scheeder MR, Wettstein HR, Kreuzer M (2005). Protein composition, plasmin activity and cheesemaking properties of cow milk produced at two altitudes from hay of lowland and high alpine origins. *J. Dairy Res.* 72: 65-74.
- Libouga DG, Womeni HM, Bitjoka L (2002). Extrait des écorces de l'*Ongokea gore*: protéolyse et conservation. *J. Cameroon Acad. Sci.* 2: 89-150.
- Libouga DG, Essia Ngang JJ, Halilou H (2005). Qualité de quelques laits fermentés camerounais. *An Int. J. Sci. Technol.* 25: 53-66.
- Madadlou A, Khosroshahi A, Mousavi ME (2005). Rheology, microstructure, and functionality of low-fat Iranian white cheese made with different concentrations of rennet. *J. Dairy Sci.* 88 : 3052-3062.
- Ministère de l'Economie et des Finances du Cameroun (2005) Note de présentation des résultats du Commerce Extérieur. Yaoundé – Cameroun.
- Nelson JH, Jensen RG, Pitas RE (1977). Pregastric esterase and other lipases – a review. *J. Dairy Sci.* 60: 327-362.
- O'Connell JE, Fox PF (2000). The two-stage coagulation of milk proteins in the minimum of the heat coagulation time-pH profile of milk: effect of casein micelle size. *J. Dairy Sci.* 83: 378-386.
- Omar MM (1985). Size distribution of casein micelles during milk coagulation. *Nahrung* 29, 119-124.
- Recueil des Normes Françaises (1980) Lait et produits laitiers - Méthodes d'analyse. Edité par l'Afnor. pp. 33-34.
- Rowland SJ (1938). The determination of nitrogen distribution of milk. *J. Dairy Res.* 9: 42-46.
- SAS User's Guide (1985). Statistics, Version - 5 Edition. Cary, NC: SAS Institute Inc.
- Somers JM, O'Brien B, Meanev WJ, Kelly AL (2003). Heterogeneity of proteolytic enzyme activities in milk samples of different somatic cell count. *J. Dairy Res.* 70 : 45-50.
- Valles E, Furret JP (1977). Etude des caillettes des bovins à l'état ruminant pour l'obtention d'extraits coagulants à base de pepsine bovine. II. Influence de la race, de l'âge et du sexe sur leur contenu enzymatique. *Lait* 61: 590-618.
- Vega-Hernandez MC, Gormez-Coello A, Villare J, Claverie-Martin F (2004). Molecular cloning and expression in yeast of caprine prochymosin. *J. Biotechnol.* 114: 69-79.
- Verdalet-Guzman I (1992). Characteristic, coagulation and cheese-making behavior of goat milk. *Arch. Latinoam. Nutr.* 42: 192-200.
- Zalaza CA, Reinheimer J (1980). Etude des additifs chimiques dans les présures liquides, leur influence dans le pouvoir coagulant et dans la présence d'une flore microbienne préjudiciable en fromagerie. *Lait* 50: 33-34.
- Zhong Q, Daubert CR, Velev OD (2004). Cooling effects on a model rennet casein gel system; part I. Rheological characterization. *Langmuir* 3. 1(20): 7399-7405.