

FACTORS AFFECTING RETENTION OF RENNET IN CURD

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ABSTRACT

The residual of calf (CR) and microbial rennet (MR) in curd playing a role in cheese ripening, depending on quantity of residual rennet retained in the curd – The effect of CaCl₂, NaCl, pH and rennet concentration on retention of rennet was studied. The results indicated that: 1)-Increasing of CaCl₂ concentrations up to 0.05% showed increasing of residual CR and MR in the curd and decreasing for whey except that of CR in the curd from buffalo's milk. 2)-Increasing of NaCl concentrations showed a reduction of residual CR and MR in the curd and whey from buffalo's and cow's milk. 3)-Reducing pH of milk showed an increase in retention of CR and MR in the curd from buffalo's and cow's milk, and reduced the retention rennet in the whey from both buffalo's and cow's milk. 4)-Increasing CR and MR concentrations as a percent appeared significant differences in the residual rennet between treatments except curd from cow's milk. Also the increasing of CR and MR concentrations as a amount of rennet unit showed increasing of residual rennet in the curd and whey from buffalo's and cow's milk.

Keywords : Ras cheese – calf and microbial rennet – buffalo's and cow's milk .

INTRODUCTION

During cheese manufacturing the added milk-clotting enzymes are distributed between the cheese curd and the whey. Retaining of the rennets in the curd affected by some factors including rennet concentrations, ionic strength, pH and other factors (Ohmiya and Sato, 1972). Although, some of the rennet enzyme may be inactivated e.g. by heat treatment and salt during cheese processing, the residual active enzyme is of main interest in cheese ripening. The amount of active milk-clotting enzyme retained in cheese will take part in breakdown of the proteins and their by have a direct influence on the texture and flavour development during ripening (Hynes *et al.*, 2001). Most of the rennet added to the cheese milk is released to the whey, but more or less 6% is retained in the curd and plays a major role in the initial proteolysis of caseins in many cheese varieties (Fox *et al.*, 2004). Residual chymosin is believed to be responsible for the initial softening of cheese through hydrolysis the Phe₂₃ - Phe₂₄ bond of α_{s1} -casein (Creamer, *et al.*, 1982).

Dulley, (1974) reported that no significant differences in the amount of residual rennet could be detected in the different cheeses, when the milling acidity was varied from 0.55% to 0.85%. Also, increasing the amount of salt added to the curd from 4.0% to 7.2% had no effect on the residual rennet. When doubling the normal amount of rennet added to the cheese milk, double the normal amount was retained in the curd, but the percentage retained remaining constant. Regardless of the amount of rennet retained, the milk clotting ability of the extracted cheese remained stable throughout

maturation. Ernstrom, (1987) concluded that the affinity of calf rennet (chymosin) for curd is markedly affected by pH of the milk, the more milk acidity more rennet retained in the curd. The same is true for porcine pepsin, but it is not true for the microbial rennets. De Roos, *et al*, (1997) noticed that the transfer of chymosin from milk into curd is due to association with para-k-casein based on electrostatic interaction and dependent on pH and ionic strength. Whereas microbial rennets will associate less and are not greatly pH dependant. Awad, (2005) found that the cheese made from salted milk contained the lowest activity of residual coagulant, while cheese made from milk pre-acidified with citric acid contained the highest activity. Bansal, *et al*, (2007) found that the retention of chymosin in cheese curd increased significantly when the pH of milk was reduced and when the ionic strength of milk was increased. The casein content of milk and the quantity of chymosin added to milk had no significant effect on the retention of chymosin in curd. The aim of this study was to investigate the effect of some factors (CaCl₂, NaCl, pH and rennet concentration) on the distribution of calf and microbial rennets between curd and whey of buffaloe's and cow's milk.

MATERIALS AND METHODS

1. Materials:

Fresh cow's and buffaloe's milk were obtained from the herds of Agriculture Faculty, Suez Canal University, Ismailia, Egypt. The low-heat skim milk powder manufacturing in USA was obtained from local market. Liquid calf rennet (45.71 RU) and microbial rennet powder (665.22 RU) (Chymax powder extra is a standardized powder a fermentation produced chymosin (FPC) produced by fermentation of *Aspergillus niger var. awamori*) were obtained from Hansen's Laboratories, Denmark. Calcium chloride was obtained from Adwic (El-Nasr pharmaceutical and chemical company, Cairo, Egypt). Sodium chloride was obtained from El-Nasr Pharmaceutical and Chemical Company, Cairo, Egypt.

2. Method of analysis:

Standard curves were prepared for each enzyme. The results were plotted in arbitrary rennet units (RU). The substrate was reconstituted skim milk (20%) containing 0.5% CaCl₂, rennet was diluted in 7.5% NaCl (5 ml diluted enzyme added to 5 ml substrate). The end-point was observed by allowing a thin film of the milk to flow from a glass rod down the side of the test-tube containing the milk. When clotting occurred, the almost invisible film broke into a number of white particles.

Rennet Activity: (Berridge, 1945):

Measuring the activities of rennets carried out by account the time (in second) required for 1 ml of the diluted enzyme to clot 10 ml of skim milk at 30 ± 0.1 °C.

Residual rennet in the curd and whey were determined according to Dulley (1974) with some modification:

Mixture of curd or cheese and 10% NaCl solution (1:1) was grounded in mortar and placed in water bath at 40 °C for 1 hr and then centrifuged at 0

°C at 3000 rpm /15 min. The aqueous phase was referred to as the cheese extract. 10 ml of extract was then combined with 10 ml of reconstituted skim milk (20%) containing 0.5% CaCl₂ and incubated at 37 °C until the appearance of the first visible coagulation. For determination residual rennet in the whey, the NaCl (7.5%) was added to the whey, then 10 ml whey was added to 10 ml reconstituted skim milk (20%) containing 0.5% CaCl₂ and incubated at 37 °C until the appearance of the first visible coagulation.

Experimental Procedure

Cow's or buffalo's milk was standardized to 4% fat, pasteurized at 70 °C and cooled directly to 37 °C. The milk divided into 4 parts (2 kg each):

- a)-The first one was divided to 4 parts (500 g each) and CaCl₂ was added at levels of 0.02 (control), 0.03, 0.04 and 0.05%, then CR (8.94 RU/500 g milk), was added.
- b)-The second part after adding CaCl₂ (0.02%) was divided to 4 parts (500 g each) and NaCl was added at levels 0 (control), 2, 4 and 6%, then CR (8.94 RU/500 g milk) was added.
- c)-To the third part CaCl₂ was added at level 0.02%, and the amount of milk was divided to 4 parts (500 g each) and the pH of milk was controlled to 6.72 (control), 6.5, 6.3 and 6.10 by diluted lactic acid, then CR (8.94 RU/500 g milk) was added.
- d)-To the fourth part, CaCl₂ was added (0.02%) at the normal pH of milk, and divided to 4 parts (500 g each) and CR was added at levels 7.31, 8.94 (control), 10.73 and 12.52 RU/500 g milk)

after curdling of all the above treatments (a, b, c and d) the curd of each treatment was cut to four pieces, placed in plastic container lined with cloth, and pressed for 6 hrs to keep the moisture a round 60%. The residual CR were determined in curd and whey.

The same above treatments were applied with MR. Three replicates were carried out for each treatment.

RESULTS AND DISCUSSION

1)-Effect of CaCl₂ concentration on retention of calf and microbial rennet in the curd and whey from buffalo's or cow's milk.

From Table (1) and Fig (1). It could be noticed that addition of CaCl₂ up to 0.05% to buffalo's milk had no significant effects on the residual CR in the curd, while these effects were significant for MR at 0.04% and 0.05%, which retained more MR as the CaCl₂ was increased.

The residual of CR and MR in buffalo's whey decreased as the CaCl₂ was increased, the decreases were significantly only at high concentration of CaCl₂.

Cow's milk curd retained more residual CR compared to that of buffalo's milk curd, at all levels of added CaCl₂, these effect was significant, while for MR the effect was significantly only at 0.04% and 0.05%.

The residual of MR in both buffalo's and cow's milk curds were quite close. The residual of CR and MR in Cow's whey showed similar trends as that of buffalo's whey which decrease with increasing CaCl₂ percent.

2)-Effect of NaCl on retention of calf and microbial rennet in the curd and whey from buffalo's or cow's milk.

Addition of NaCl up to 6% resulted in a decrease in retention of both CR and MR in buffalo's and cow's curd and these effect was significant. (Table 2 and Fig 2). Similar observation was reported by Awad (2005) found that soft cheese made from salted milk contained low activity of residual coagulants.

There are slight differences could be noticed on the effect of NaCl on retention of both CR and MR in buffalo's and cow's curd. The residual CR and MR of buffalo's whey were significantly reduced, as the salt concentration was increased. Buffalo's and cow's whey resulted from CR and MR was affected by salt in a similar way. The NaCl has a direct effect on the rennet activity which retard the milk clotting time, and accordingly the activity of residual rennet (Fox & Walley, 1971 and Gouda, 1987).

3)-Effect of pH on retention of CR and MR in the curd and whey from buffalo's or cow's milk.

It could be noticed that from Table (3) and Fig (3) the retention of CR and MR in both buffalo's and cow's curd markedly increased as the milk pH decreased. De Roos, *et al.*, (1997) said that transfer of chymosin from milk into curd is due to association with para-k-casein base on electrostatic interaction. This association increases with lower pH and ionic strength. Holmes, *et al.*, (1977) and Ernstrom, (1987) reported that association of chymosin is highly dependent on pH and ionic strength.

From the same table and figure also, we could noticed that the effect of decreasing pH milk on increasing retention of both coagulants in buffalo's and cow's curd were significant. However, cow's curd was less affect by the milk pH than that of the buffalo's one, which retained less CR and MR comparing to that of buffalo's curd at all pH levels. Also, reducing cow's milk pH resulted in higher retention of MR in cow's curd than that of CR. This was not confirmed by Ernstrom (1987) and Garnot *et al.*, (1987) they reported that the affinity of CR (chymosin) for curd is markedly affected by pH of the milk, more acid in milk resulted more rennet in the curd, and they added that these is not true for MR. Also, the increase of CR and MR retention could be partially attributed also to the increase in activity of these coagulants by reducing the pH of the curd and whey. Lowering the milk pH from 6.72 to 6.10 decreased the residual of both CR and MR in the resultant whey from buffalo's and cow's milk, and this effect was more pronounced in cow's whey, which retained much more coagulant

4)-Effect of rennet concentration on retention of CR and MR in the curd and whey from buffalo's or cow's milk

Increasing the CR and MR concentration had no significant effect on its retention in cow's milk curd as a percentage of the initial added rennet unit (RU) (Table 4 and Fig 4). In other words the quantity retained of both rennets in the curd increased by increasing the added RU (Table 5 and Fig 5), but the ratio of the retained to the initial rennet remained constant (Table 4).

T1-2-3

Fig (1)-Effect of CaCl_2 concentration on retention of calf and microbial rennet in the curd and whey from buffaloe's or cow's milk.

RCR, Residual calf rennet; RMR, Residual microbial rennet
A, Residual CR in curd; B, Residual CR in whey; C, Residual MR in curd and D, Residual MR in whey

Fig (2): Effect of NaCl on retention of CR and MR in the curd and whey from buffaloe's or cow's milk

RCR, Residual calf rennet; RMR, Residual microbial rennet

A, Residual CR in curd; B, Residual CR in whey; C, Residual MR in curd and D, Residual MR in whey

Fig (3): Effect of pH on retention of CR and MR in the curd and whey from buffaloe's or cow's milk

RCR, Residual calf rennet; RMR, Residual microbial rennet
A, Residual CR in curd; B, Residual CR in whey; C, Residual MR in curd and D, Residual MR in whey

T4-5

Fig (4): Effect of rennet concentration on retention of CR and MR in the curd and whey from buffaloe's or cow's milk

RCR, Residual calf rennet; RMR, Residual microbial rennet

A, Residual CR in curd; B, Residual CR in whey; C, Residual MR in curd and D, Residual MR in whey

Fig. (5): Effect of rennet concentration on retention of calf and microbial rennet as a quantity of rennet unit (RU) in the curd and whey from buffaloe's or cow's milk

RCR, Residual calf rennet; RMR, Residual microbial rennet
A, Residual CR in curd; B, Residual CR in whey; C, Residual MR in curd and D, Residual MR in whey

Similar observation was reported by Dullely (1974) who mentioned that when double the normal amount of rennet was added to the cheese milk, double the normal amount was retained in the curd, but the percentage retained remaining constant. Also, De Roos *et al.*, (1997) notices that association of chymosin with curd increased with high chymosin concentration. Ernstrom, (1987) established that when one third normal amount of rennet was used, the total amount of residual enzyme in the curd was lower than normal.

Table (4) revealed that the retention percentage of MR in both buffaloe's and cow's curd was higher than CR at all rennet concentration. The effect of concentration of CR and MR on the percentage residual rennet in the whey of buffaloe's and cow's milk was not noticeable.

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العوامل المؤثرة علي احتفاظ الخثرة بالمنفحة

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- لبقايا المنفحة الحيوانية والميكروبية في الخثرة دور في تسوية الجبن - ولقد تمت دراسة تأثير كلا من كلوريد الكالسيوم ، وكلوريد الصوديوم ، الأس الهيدروجيني وتركيز المنفحة الحيوانية والميكروبية علي احتفاظ الخثرة والشرش بالمنفحة في اللبن الجاموسى والبقرى وأظهرت النتائج الآتي:
- (١)- أدت زيادة تركيز كلوريد الكالسيوم إلي ظهور اختلافات معنوية ملحوظة علي بقايا المنفحة الحيوانية والميكروبية بالخثرة والشرش باستثناء بقايا المنفحة الحيوانية بخثرة اللبن الجاموسى
 - (٢)- أدت زيادة تركيز كلوريد الصوديوم إلي خفض نسبة المنفحة الحيوانية والميكروبية بالخثرة والشرش الناتجين من اللبن الجاموسى والبقرى
 - (٣)- أدت خفض الأس الهيدروجيني للبن إلي زيادة نسبة احتفاظ خثرة اللبن الجاموسى والبقرى بالمنفحة الحيوانية والميكروبية والى نقصان نسبة المنفحة في الشرش الجاموسى والبقرى
 - (٤)- أدت زيادة تركيز المنفحة الحيوانية والميكروبية كنسبة مئوية إلي ظهور اختلافات في بقايا المنفحة بين المعاملات باستثناء خثرة اللبن البقرى ، كما أدت زيادة تركيز المنفحة الحيوانية والميكروبية (ككمية) إلي زيادة بقايا المنفحة بالخثرة والشرش الجاموسى.

Table (1): Effect of CaCl₂ on retention of calf and microbial rennet in the curd and whey from buffalo's or cow's milk

Treatment	Buffaloe's milk				Cow's milk			
	Curd		Whey		Curd		Whey	
	Residual CR (%)	Residual MR (%)	Residual CR (%)	Residual MR (%)	Residual CR (%)	Residual MR (%)	Residual CR (%)	Residual MR (%)
CaCl ₂ %	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0.02*	25.09 ^a ± 0.95	31.32 ^c ± 1.58	72.89 ^a ± 1.07	64.84 ^{ab} ± 1.46	28.20 ^c ± 1.03	32.74 ^b ± 0.84	70.32 ^a ± 1.20	64.39 ^a ± 1.25
0.03	25.21 ^a ± 0.79	33.56 ^{bc} ± 1.43	71.44 ^a ± 0.90	65.62 ^a ± 0.85	30.83 ^b ± 1.07	33.18 ^b ± 0.91	67.45 ^b ± 1.10	63.61 ^a ± 0.90
0.04	25.80 ^a ± 1.06	35.50 ^{ab} ± 0.84	67.64 ^b ± 1.21	64.54 ^{ab} ± 0.89	31.65 ^b ± 0.89	36.28 ^a ± 0.78	65.65 ^b ± 1.26	62.64 ^a ± 1.21
0.05	25.21 ^a ± 1.18	36.73 ^a ± 1.29	65.10 ^c ± 1.18	62.42 ^b ± 1.34	34.49 ^a ± 0.85	37.10 ^a ± 0.90	62.57 ^c ± 1.13	60.16 ^b ± 1.60
LSD 5%	Ns	2.469	2.067	2.199	1.815	1.619	2.211	2.379

* control; CR, calf rennet; MR, microbial rennet; The same letters means, no significant differences; The different letters means, significant differences; (P>0.05). SD, standard deviation.

Table (2): Effect of NaCl on retention of calf and microbial rennet in the curd and whey from buffalo's or cow's milk

Treatment	Buffaloe's milk				Cow's milk			
	Curd		Whey		Curd		Whey	
	Residual CR (%)	Residual MR (%)						
NaCl%	Mean ± SD							
0*	24.27 ^a ± 1.21	32.06 ^a ± 1.06	72.33 ^a ± 0.67	64.62 ^a ± 1.24	27.70 ^a ± 1.23	29.23 ^a ± 0.84	75.80 ^a ± 0.80	69.40 ^a ± 1.15
2	16.42 ^b ± 0.74	19.52 ^b ± 0.72	56.26 ^b ± 1.23	56.26 ^b ± 1.14	16.70 ^b ± 1.89	18.87 ^b ± 0.95	57.41 ^b ± 1.17	54.49 ^b ± 1.16
4	13.95 ^c ± 1.31	14.91 ^c ± 1.09	49.41 ^c ± 1.48	51.64 ^c ± 1.12	13.91 ^c ± 1.30	16.16 ^c ± 0.84	51.51 ^c ± 1.22	52.67 ^b ± 1.15
6	10.98 ^d ± 0.43	11.97 ^d ± 1.07	43.17 ^d ± 0.83	43.81 ^d ± 1.26	11.47 ^c ± 0.79	14.50 ^d ± 0.62	43.41 ^d ± 1.04	41.28 ^c ± 0.99
LSD 5%	1.862	1.879	2.070	2.238	2.554	1.543	2.013	2.099

* control; CR, calf rennet; MR, microbial rennet; The same letters means, no significant differences; The different letters means, significant differences; (P>0.05). SD, standard deviation.

Table (3): Effect of pH on retention of calf and microbial rennet in the curd and whey from buffalo's or cow's milk

Treatment	Buffaloe's milk				Cow's milk			
	Curd		Whey		Curd		Whey	
	Residual CR (%)	Residual MR (%)						
pH values	Mean ± SD							
6.72*	24.68 ^d ± 0.93	31.39 ^d ± 1.57	74.80 ^a ± 0.95	67.26 ^a ± 0.68	26.28 ^d ± 1.02	29.19 ^d ± 1.14	75.58 ^a ± 1.13	71.03 ^a ± 1.01
6.50	33.19 ^c ± 1.16	39.97 ^c ± 1.12	68.13 ^b ± 1.39	65.25 ^a ± 1.51	30.84 ^c ± 1.15	33.74 ^c ± 0.84	72.44 ^b ± 1.16	67.07 ^b ± 1.18
6.30	45.97 ^b ± 1.07	53.76 ^b ± 0.79	60.52 ^c ± 1.15	60.85 ^b ± 1.07	41.13 ^b ± 1.30	38.93 ^b ± 1.01	65.40 ^c ± 0.72	64.05 ^c ± 1.23
6.10	57.64 ^a ± 1.10	60.59 ^a ± 0.99	51.67 ^d ± 1.29	50.27 ^c ± 1.14	51.75 ^a ± 0.84	55.52 ^a ± 0.80	56.19 ^d ± 1.45	58.32 ^d ± 0.86
LSD 5%	2.012	2.170	2.273	2.149	2.053	1.797	2.151	2.032

* control; CR, calf rennet; MR, microbial rennet; The same letters means, no significant differences; The different letters means, significant differences; (P>0.05). SD, standard deviation.

Table (4): Effect of rennet concentration on retention of calf and microbial rennet as a percent in the curd and whey from buffalo's or cow's milk

Treatment	Buffaloe's milk				Cow's milk			
	Curd		Whey		Curd		Whey	
	Residual CR (%)	Residual MR (%)	Residual CR (%)	Residual MR (%)	Residual CR (%)	Residual MR (%)	Residual CR (%)	Residual MR (%)
RU	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
7.31	24.51 ^b ± 1.10	29.15 ^b ± 0.96	69.45 ^b ± 1.27	69.31 ^a ± 0.63	26.40 ^a ± 0.76	29.20 ^a ± 0.67	69.43 ^c ± 0.92	70.00 ^a ± 1.10
8.94*	25.17 ^b ± 1.36	33.15 ^a ± 0.96	74.01 ^a ± 1.13	67.56 ^{ab} ± 0.73	27.48 ^a ± 1.38	29.01 ^a ± 1.07	75.10 ^a ± 1.63	70.00 ^a ± 1.15
10.73	26.34 ^{ab} ± 0.70	32.86 ^a ± 1.12	74.81 ^a ± 1.14	67.59 ^{ab} ± 1.18	27.75 ^a ± 1.09	28.57 ^a ± 0.57	72.48 ^b ± 0.79	69.90 ^a ± 1.10
12.52	28.04 ^a ± 0.96	32.91 ^a ± 1.05	73.37 ^a ± 0.77	66.55 ^b ± 0.87	27.78 ^a ± 0.78	28.51 ^a ± 0.56	74.53 ^{ab} ± 0.91	71.86 ^a ± 0.88
LSD 5%	1.988	1.928	2.057	1.653	Ns	Ns	2.096	Ns

* control; CR, calf rennet; MR, microbial rennet; The same letters means, no significant differences; The different letters means, significant differences; (P>0.05). SD, standard deviation.

Table (5): Effect of rennet concentration on retention of calf and microbial rennet as a quantity of rennet unit (RU) in the curd and whey from buffalo's or cow's milk

Treatment	Buffaloe's milk				Cow's milk			
	Curd		Whey		Curd		Whey	
	Residual CR (amount)	Residual MR (amount)						
RU	Mean							
7.31	1.79 ^d ± 0.08	2.13 ^d ± 0.07	5.08 ^d ± 0.09	5.07 ^d ± 0.05	1.93 ^d ± 0.06	2.13 ^d ± 0.05	5.08 ^d ± 0.07	5.12 ^d ± 0.08
8.94*	2.25 ^c ± 0.12	2.96 ^c ± 0.09	6.62 ^c ± 0.10	6.04 ^c ± 0.07	2.46 ^c ± 0.12	2.59 ^c ± 0.10	6.71 ^c ± 0.15	6.26 ^c ± 0.11
10.73	2.83 ^b ± 0.08	3.53 ^b ± 0.12	8.03 ^b ± 0.12	7.25 ^b ± 0.13	2.98 ^b ± 0.12	3.07 ^b ± 0.06	7.78 ^b ± 0.09	7.50 ^b ± 0.12
12.52	3.51 ^a ± 0.12	4.12 ^a ± 0.13	9.19 ^a ± 0.10	8.33 ^a ± 0.11	3.48 ^a ± 0.10	3.57 ^a ± 0.07	9.33 ^a ± 0.11	9.00 ^a ± 0.11
LSD 5%	0.192	0.197	0.195	0.177	0.192	0.133	0.203	0.197

* control; CR, calf rennet; MR, microbial rennet; The same letters means, no significant differences; The different letters means, significant differences; (P>0.05).