

Proteolytic Activity of Different Rennets on Cow and Buffalo Milk

K. G. Upadhyay, S. H. Vyas, A. J. Pandya,
J. N. Patel, and H. K. Desai

Department of Dairy Technology, Sheth M.C.
College of Dairy Science, GAU, Anand
Campus, Anand

In recent years the enzymes derived from *Mucor pusillus* Lindt and *Mucor miehei* have found widespread application for cheese-making. In the previous publication (Upadhyay *et al.* in press) the commercial fungal rennets, namely, Rnnilase and Meito rennet derived from *Mucor miehei* and *Mucor pusillus* Lindt respectively, were compared with calf rennet using them alone or as their mixtures for their influence on clotting time, curd characteristics and optimum dosage for Cheddar cheese-making from buffalo milk. In this paper the same fungal rennets and their mixtures are compared for their proteolytic activity on buffalo and cow skim milks using calf rennet as control.

Fresh cow and buffalo milks (pH adjusted to 6.7) were used as substrate. The rennet solutions were standardized to same coagulation power by suitably diluting the rennets with distilled water. One millilitre of such diluted rennet solution coagulated 50 ml of fresh buffalo skim milk in 10 min at 32° C. The proteolytic activity was measured in terms of increase in non-protein-nitrogen (NPN) soluble in 12% tri-

chloroacetic acid (TCA) at different time intervals.

Fifty millilitres of buffalo skim milk were transferred to 5 conical flasks for each rennet solution and placed in a water bath at 32° ± 0.1° C. When the temperature was attained, 1 ml of each of the diluted rennet solution was added and mixed well. After 0, 30, 60, 90 and 120 min of incubation, 51 ml of 24% TCA were added to each flask, mixed well and the contents were filtered through Whatman No. 42 filter paper. The sample at time zero was obtained by adding 1 ml of denatured rennet solution to 50 ml skim milk. The denaturation was carried out by heating the diluted rennet solutions at 95° C for 10 min. The NPN of the filtrate was determined in duplicate by the Kjeldahl method and expressed as milligram per cent increase over the initial at zero time. Identical set of experiment was run using cow skim milk as substrate.

The results of the proteolytic activity of different rennets carried over a period of 120 min are presented in Table-1. It is observed that in both milks the increase in NPN for all coagulants, except for rennet C and CR, showed a sharp rise upto 60 min of the enzyme action followed by gradual rise upto the end of incubation time. In case of C rennet, after an initial rise in the first 60 min, the level of NPN remained constant until 120 min in both milks. Against this, CR rennet

TABLE 1

Proteolytic activity of different rennets on buffalo and cow skim milk at 32° C.

Milk	Treatment in minutes	Increase in NPN, mg/100 ml					
		C	M	R	CM	CR	MR
Buffalo (pH 6.7)	0	—	—	—	—	—	—
	30	1.68	2.24	2.24	2.24	2.24	2.24
	60	2.80	2.80	2.80	2.80	2.80	2.80
	90	2.80	2.80	3.36	2.80	2.80	3.36
	120	2.80	3.36	3.36	3.36	2.80	3.36
Cow (pH 6.7)	0	—	—	—	—	—	—
	30	3.92	5.60	5.04	6.16	5.60	5.60
	60	5.60	6.72	5.60	6.72	5.60	6.16
	90	5.60	6.72	6.16	6.72	5.60	6.72
	120	5.60	7.28	7.28	7.28	6.16	7.28

Average of 3 trials.

C : Calf Rennet CM : Calf + Meito (1 : 1, w/w)

M : Meito CR : Calf + Rennilase (1 : 1, w/v)

R : Rannilase MR : Meito + Rennilase (1 : 1, w/v)

showed slightly different pattern of release of NPN after 90 min incubation time in cow skim milk, showing some increase in NPN. In buffalo milk, it behaved alike calf rennet.

The data further revealed that the increase in NPN was slower in buffalo skim milk compared to cow milk, irrespective of the type of rennet used. This is in agreement with the results of Ganguli *et al.* (1964), Gupta and Ganguli (1965), Angelo (1976) and Abdou *et al.* (1976), who reported that irrespective of the type of rennet, proteolytic activity is faster and more with cow milk than with buffalo milk. Among the various rennets used, C rennet showed the least proteolytic activity both in cow milk (3.92 mg NPN increase) and buffalo milk (1.68 mg NPN increase).

On the other hand, Mucor rennets, namely M and R when used alone showed higher proteolytic activity than calf rennet in both the milks over a

period of 120 min. The CM and MR combinations were also equally proteolytic in both milks at 120 min., showing that the CM and MR combinations are compatible. However, CR combination had lower proteolytic activity than R alone in both the milks over a period of 120 min. This could possibly be due to incompatibility of C and R enzymes in association. Such incompatibility—one enzyme affecting the stability of another enzyme in association—has been reported by Richardson *et al.* (1967).

The above findings of the study reveal that fungal rennets and their mixtures, except CR combination are more proteolytic than calf rennet. This is in agreement with the results of Richardson *et al.* (1967) and Philippos and Christ (1976) who found these Mucor rennets, namely, *M. pusillus* Lindt and *M. miehei* to be more proteolytic than calf rennet in cow milk.

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