

THE ACTION OF RENNET ON MILK.

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DURING the renneting process in the manufacture of Cheddar cheese a retardation of the time of coagulation has often been noticed though the acidity had reached the required strength before the rennet was added to the milk. The cause or causes of this retardation are of great technical importance as they lead to infection of the milk with consequent production of taints. As the two main factors of coagulation are (i) the acidity of the milk when renneted, and (ii) the calcium salts of the milk, we approached the inquiry from these two points and tried to answer the following questions:—

(1) Is *all* the acidity of the milk produced on standing due to the formation of lactic acid?

(2) Would it be possible to produce a real *Cheddar Cheese Curd* if to the milk the necessary amount of lactic was added before renneting?

(3) How far is the coagulation time retarded on dialysis of the calcium salts of the milk?

As regards the first question our inquiry has led us to believe that the total acidity is not actually due to lactic acid, and this is brought out in Table I which gives the amounts of lactic sugar in milk at different times

TABLE I.

Time	Lactose	Lactic acid		
		Found	Increase of acidity	
			Found	Calc. for max. 4 mols
hours	grams	grams	grams	grams
0	3.762	0.926	—	—
6	3.224	1.723	0.796	0.538
19	2.965	2.764	1.838	0.797
27	2.249	3.286	2.360	1.513

together with the acidity. The figures are expressed for 100 c.cs. milk. The sugar estimations were carried out according to Ritthausen¹, and the acidity estimated by titration with N/10 caustic soda, using phenol phthalein as an indicator.

TABLE II.

Temperature: 33—35° C.

Time	Acidity expressed in N/1000 KOH	Increase of acidity
mins.	c.cs.	c.cs.
0	0·4	—
30	11·40	11·0
60	17·80	17·4
90	24·60	24·2
120	34·20	33·8
150	41·70	41·3
180	68·40	68·0
210	87·7	87·3
240	94·8	94·4
270	102·6	102·2

TABLE III.

Temperature: 24° C.

Time	Acidity expressed in N/1000 KOH	Increase of acidity
mins.	c.cs.	c.cs.
0	slightly alkaline	—
30	8·8	—
60	14·8	6·0
90	23·4	14·6
120	34·7	25·9
150	43·1	34·3
180	64·3	55·5
210	78·4	69·6
240	94·6	85·8

These results, which are only one instance taken from three similar experiments, obviously indicated that the increase of the acidity of milk was not due to the formation of lactic acid only, and this was confirmed by the following experiments. To a solution of caseinogen made as neutral as possible by Hammarsten's method, rennet was added (3 g. caseinogen in 50 c.cs. Ca(OH)₂ and phosphoric acid and 0·2 c.c. of a 1 per cent.

¹ Ritthausen, *Journ. f. prakt. Chemie*, II. 15. 329 [1877].

solution of rennet were used). The increase of acidity was estimated for 5 c.cs. with N/1000 KOH solution and phenol phthalein used as an indicator. The estimations were made at 33—35° C. and 24° C. The results are expressed in Tables II and III.

We then further studied the effect of rennet in the presence of lactic acid in order to see whether this acid does or does not stimulate a higher production of acidity during the action of rennet on caseinogen. To a caseinogen solution we therefore added 25 c.cs. of a 1/40 per cent. lactic acid solution and carried out the estimation as before. Table IV gives the results.

TABLE IV.
Temperature: 24° C.

Time	Acidity expressed in N/1000 KOH	Increase of acidity
mins.	c.cs.	c.cs.
0	44.6	—
30	64.8	20.2
60	79.2	34.6
90	94.7	50.1
120	127.2	82.6
150	147.4	102.8
180	189.7	145.1

These results show that rennet is capable of producing acidity from caseinogen and that this process is stimulated by the addition of lactic acid. In the present position of the investigation it is difficult to understand how the acidity is actually produced, and further work on these lines must be carried out before we are in a position to offer a definite explanation.

An increase of acidity having been fully established it was thought possible that lactic acid might be used as a "starter" in the renneting of milk, since we had found that it apparently acted as a stimulant in the production of acidity. The advantages of such a material as a "starter" are obvious. To 10 gallons of fresh milk 10 c.cs. *pure Kahlbaum's lactic acid* and 23 c.cs. rennet were added. At the time of renneting the acidity of the milk was approximately 0.14 per cent., which is the required acidity in the renneting of milk for Cheddar cheese. Soon after the rennet was added the milk coagulated and formed a curd consisting of *non-adhering parts quite unlike the curd formed under ordinary conditions*. At that moment the acidity was 0.17 per cent. and did not

change for 9 hours, which is also quite abnormal. It afterwards progressed slowly in 12 hours to 4·7 per cent. and in 13 hours to 7·2 per cent., whereas under normal conditions this acidity would have been reached in 5—7 hours. The cheese thus obtained consisted of a hard crummy mass and was certainly of a very inferior type. We believe this was due to the fact that during the process the caseinogen had been precipitated out by lactic acid and not transformed into casein. In the light of our previous experiments it must be concluded that the acidity of milk before renneting is certainly not due to lactic acid *only*, for were this acid present in the milk to the same extent when usually renneted it would precipitate out the caseinogen and hence prevent its transformation into casein.

As the retardation of the coagulation could have been explained by the fact that the milk did not contain enough calcium, we estimated the coagulation time of milk before and after dialysis. However, as it will be seen from Table V, it is quite impossible to conclude that this can be explained on such lines. The calcium estimations were carried out in the following way: the milk or dialysed milk was evaporated to dryness and the calcium estimated in the ash by precipitating with oxalic acid, washing the precipitate carefully and finally transferring to a beaker by means of a jet of water and titrating with potassium permanganate in the presence of sulphuric acid.

TABLE V.

	Non-Dialysed Milk			Dialysed Milk		
	Acidity	Calcium in 10 c.cs.	Time of coagn. of 25 c.cs.	Time of Dialysis	Calcium in 10 c.cs.	Time of coagn. of 25 c.cs.
	per cent.	gms. CaO	mins.	hours	gms. CaO	mins.
1	0·21	0·0116	31	4	0·0102	57
2	0·22	0·0112	25	4	—	38
3	0·16	0·0100	30	4	0·0098	46
4	0·16	0·0112	27	4	0·0102	34
5	0·19	0·0110	20	4	0·0098	10
6	0·16	0·0118	31	4	0·0108	32
7	0·16	0·0116	20	4	0·0088	25
8	0·17	0·0110	22	19	0·0082	56
9	0·16	0·0116	30	19	—	—
10	0·17	—	23	23	0·0082	50

If we analyse the results in Table V we find that there is no relationship between the length of the time the dialysis was allowed to

proceed and the loss of calcium, and that there is further no relationship between the loss of calcium and the retardation of the time of coagulation.

Though this investigation does not in any way throw light on the reason of the retardation of the coagulation, it has at any rate shown that this is not entirely due to the acidity or to the calcium salts of the milk as is usually assumed.

Conclusions.

- (1) The acidity of milk is not due entirely to lactic acid, but partly to some product produced from caseinogen.
- (2) Pure lactic acid cannot be used as a starter, though it stimulates the production of acid from caseinogen.
- (3) The retardation of the time of coagulation with rennet is not entirely dependent on the calcium salts.

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