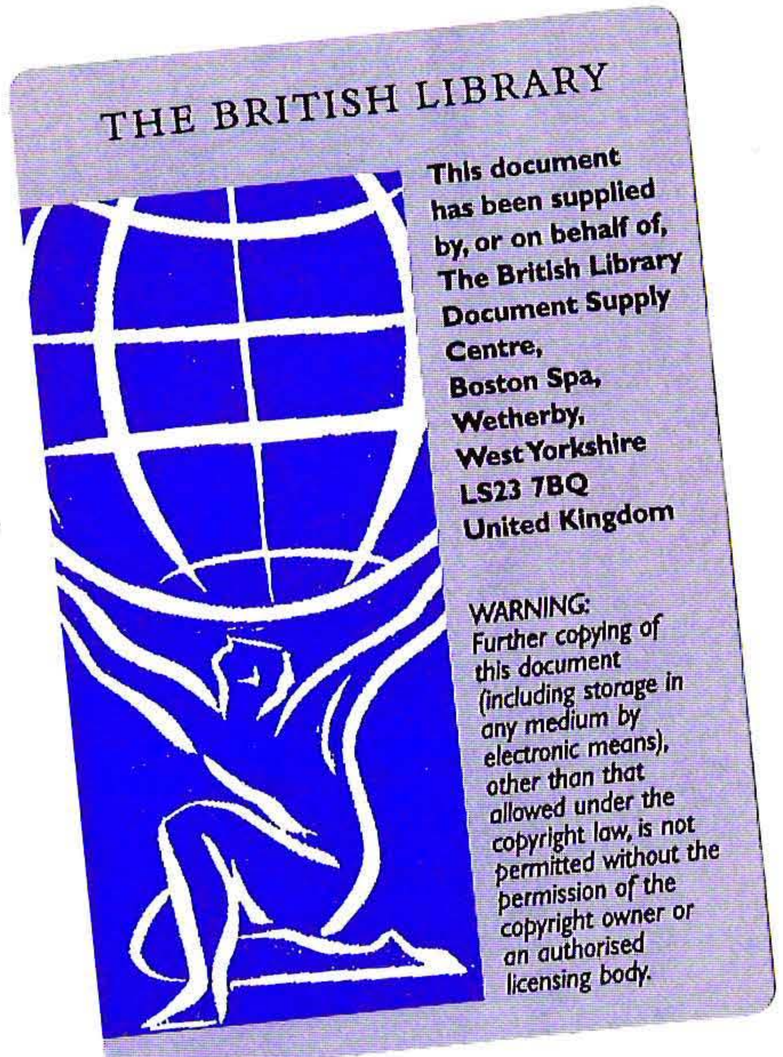

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602. EXPERIMENTS IN CHEESEMAKING WITHOUT STARTER

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(With 3 Figures)

For the preparation of good Cheddar, Cheshire and similar cheese a correct rate of acidification and degree of acidity is essential. Therefore the faults to which the starter organisms are prone may have severe consequences. For this reason it has often been suggested that alternative methods of acidification should be explored. A successful method would, at one stroke, cut the Gordian knot of bacteriophage, antibiotics, winter slowness and other difficulties.

According to Van Slyke & Publow (1) previous attempts to use dilute acid in place of starters had not been adequately controlled. Hucker & Marquardt (2) claimed to be able to produce normal cheese by using lactic acid in place of starter, but the experience of others as well as our own experiments show that the normal cheesemaking implied by Hucker & Marquardt cannot be achieved simply by the addition of acid. These workers were, moreover, equally successful in producing cheese without either starter or lactic acid, and this was doubtless due to their use of raw milk with the relatively high count of 500,000–1,000,000 bacteria/ml. It appears that their results were due to the initial flora of the milk acting as starter, and that therefore they do not indicate the successful use of artificial acidification.

More recently Mulder & Radema (3), making Edam cheese under difficulties of fuel shortage, added calcium chloride and hydrochloric acid to milk to improve its clotting properties. They continued the use of starter, but the added calcium and hydrogen ions permitted clotting and syneresis to take place at relatively low temperatures. The amount of hydrochloric acid they needed was less than one-tenth of that necessary to secure sufficient acidity for the proper ripening of English hard pressed cheese.

The experiments reported in this paper fall into two sections; the first concerns the preparation, without the use of a starter culture, of a curd with satisfactory physical properties, and the second concerns the improvement in flavour of the ripened product.

MATERIALS AND METHODS

Milk. A mixture of morning and evening milk was used in Exps. 1–4. Bulked morning milk was employed in the remaining experiments. In all cases the bacterial content was about 5000 organisms/ml. or less.

Pasteurization. Two methods were employed. The first (Exps. 19, 21, 22) involved passing warmed milk at about 500 ml./min. through two glass condensers, whose outer jackets were steam-heated, so that a maximum temperature of 68° was reached. After 1 or 2 sec. at this temperature the milk was cooled by passing it through coils immersed in cold water. Alternatively (Exp. 24) the milk was heated to 57° over 20 min., held at 57° for 3 min. and cooled.

Cheesemaking. The cheeses were made on a 14 gal. scale using a method based on the Cheddar and Cheshire processes. In most cases the curd was milled finely as for Cheshire cheese so that extra acid could easily be incorporated at this stage. Modifications were introduced as thought desirable. The cheeses were ripened at 55° F.

Estimation of quality of cheese. Cores were taken at intervals during the ripening period and tasted by a small group of workers (usually six) who were accustomed to assessing the flavour of cheese. Any cheeses which were thought to be satisfactory were then distributed to about twelve people who were not necessarily experienced in cheese-tasting.

EXPERIMENTAL

The techniques used and some of the observations made are recorded in Tables 1 and 2. The schedule of Table 3 is provided to form a concrete example of the process which was most frequently used in the later experiments, and to provide, together with Tables 1 and 2, a background for the notes which follow.

Preparation of a curd resembling that of Cheddar cheese. Normally the curd of Cheddar cheese before pressing has a pH of about 5·5, and this drops quickly to below 5 as the starter organisms continue to multiply and produce lactic acid. In the absence of starter organisms it was reasonable at first to try acidifying cheese-milk with lactic acid to control the growth of unwanted flora, e.g. coliforms.

In Exp. 1 commercial lactic acid (200 ml.) diluted with an equal volume of water was added during 2 min. with rapid agitation to milk at 86° F. to give a pH of about 5·4. A normal quantity of rennet was then added during 15 sec.; clotting took place after only 2 min. because of the low pH, and the curd was cut after 15 min. The usual Cheddar process was then followed. A silky, weak-bodied curd was obtained oozing whey under manual pressure. Under tension the curd particles were elastic and did not break cleanly like a normal curd. Little improvement in body took place on Cheddaring.

In an attempt to get a drier curd (Exp. 2) sufficient lactic acid to give a pH of only 5·8 in the milk was added before renneting; calcium chloride ($CaCl_2.6H_2O$, 200 g.) in aqueous solution was also added during scald to diminish the loss of calcium from the acidified curd in the hope that its physical properties would thereby be improved. Some improvement indeed took place. The Cheddared curd had a pH of 5·9, and it was necessary to consider how best to reduce this value so that it would reach about 5·0 after pressing. Gluconic acid lactone was chosen for this purpose; it hydrolyses to the free acid at a convenient rate (Fig. 1), it is readily available, non-toxic, and free from other objectionable properties such as insolubility of calcium salts and unpleasant taste. The lactone (165 g.) was added with the normal quantity of salt to the finely milled curd just before pressing.

Whether the improvement in curd properties in Exp. 2 resulted from the higher pH at renneting or from the addition of the calcium chloride was explored in Exp. 3. Here calcium chloride was again added, but the pH was adjusted to about 5·4 before the addition of rennet. The inferior curd obtained suggested that in the former experiment the higher pH at renneting was the more important factor and a similar pH was consequently employed in succeeding work.

In Exp. 4 hydrochloric acid was used instead of lactic acid for reasons of economy. Its use did not apparently affect the physical properties of the curd. The higher pH at

Table 1

Exp. no.	Additions to milk: Lactic acid (ml.)	Hydrochloric acid (ml. 3·3 N)	Gluconic acid lactone (g.)	Calcium chloride (g.)	Scald: Renneting (pH)	Calcium chloride (g.)	Hydrochloric acid (ml. 2 N)	Time (min.)	Final temp. (° F.)	Pitching: pH whey	pH curd	Addition to curd: Gluconic acid lactone (g.)	Salt (oz.)	Comments about curd
1	200	—	—	—	5·35	—	—	—	—	5·6	5·30	—	7	Silky. Weak body. Rubbery
2	133	—	—	—	5·82	200	—	35	100	—	5·90	165	4	Firmer than Exp. 1. Rubbery
3	180	—	—	—	5·34	100	—	—	—	—	5·30	—	—	Lacking body. Curd membrane elastic
4	—	180	—	200	5·76	—	90	—	—	5·48	—	165	5	Similar to Exp. 2
5	—	240	—	—	5·85	200	—	—	—	—	—	165	4	Good body
6	—	240	—	—	5·80	—	—	—	—	—	—	165	5	Similar to Exp. 5
7	—	240	—	—	5·95	—	105	40	—	5·57	5·82	165	5	Too shotty
8	—	270	—	—	5·80	—	120	32	98	5·46	5·80	165	5	Very shotty
9	—	255	—	—	5·88	—	120	28	94	5·73	—	165	5	Too wet
10	—	255	—	—	5·96	—	120	45	94	5·70	—	165	5	Shotty. Wet inside particles
11	—	255	—	—	—	—	120	63	86	—	—	165	5	Shotty and would not mat
12	—	240	—	—	6·02	—	105	43	98	5·78	6·01	165	5	Shotty, but matted
13	—	240	—	—	6·02	—	105	70	98	5·74	6·00	165	5	Slightly shotty, matted well, tore well
14	—	66	—	—	6·52	—	150	40	98	6·0	—	215	5	Shotty, inferior to normal curd
15	—	60	160	—	6·47	—	105	60	98	5·68	5·90	165	5	Normal at cutting. Tough at milling
16	—	60	160	—	6·46	—	—	68	98	5·92	6·06	165	5	Pitched normally. Slightly brittle
17	—	60	360	—	6·60	—	—	40	98	—	5·84	165	5	Silky. Good hot-iron test

Table 2

Exp. no.	Additions to cheese milk: Hydrochloric acid (ml. 3·3 N)	Gluconic acid lactone (g.)	Trypsin (g.)	Acetic acid (ml. 5 N)	*L. casei* 2d. 30° (ml.)	*L. brevis* 2d. 30° (ml.)	Scald: Hydrochloric acid (ml. 2 N)	Time (min.)	Final temp. (° F.)	Whey drawing: Whey	Curd	Gluconic acid lactone (g.)	Salt (oz.)	Comments about curd
18	60	160	—	—	120	—	105	50	98	5·6	6·02	165	5	Normal
19	60	350	—	—	70	—	—	46	98	—	—	165	5	Hot-iron test good. Rough tear
20	60	350	1·0	—	—	—	—	54	98	—	—	165	5	Hot-iron test very good. Rough tear
21	—	350	1·0	60	120	—	—	45	98	—	—	165	5	Very buttery odour at milling
22	60	360	1 l. tryptic digest	—	20	—	—	43	98	5·55	—	165	5	Very good at cutting stage. Handled well at pitching
23	60	360	—	—	40	40	—	46	98	5·55	—	165	5	Some floated at pitching
24	60	360	—	—	5	5	—	45	98	—	5·78	165	5	Slight floating at pitching

rennetting had, however, introduced the difficulty of obtaining a sufficiently low pH at the milling stage. To overcome this, hydrochloric acid (2 N, 150 ml.) was added slowly during the scalding. The curd behaved normally until near the end of the scald when it suddenly became 'shotty', presumably because the main action of acid had been on the outer surfaces of the curd particles. Subsequent work (Exp. 5–13) consisted mainly in various modifications of the process of Exp. 4 (see Table 1). No modification gave a pitched curd with normal properties.

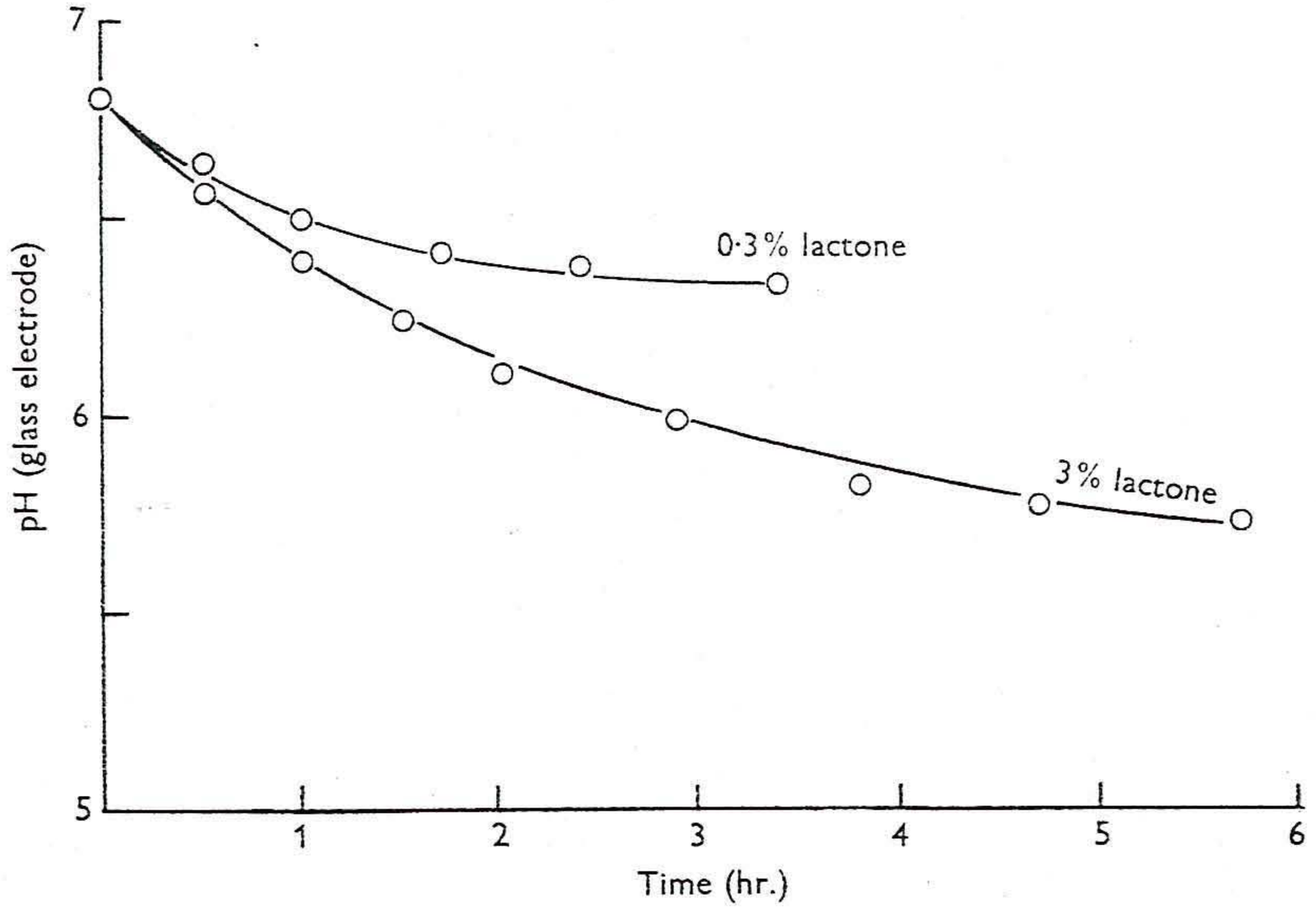

Fig. 1. Rate of hydrolysis of gluconolactone in milk.

Table 3. *Schedule of operations in making cheese without starter*

Time after beginning hr.	Time after beginning min.	Approx. period of time for operation	Operation
0	0	12 min.	4·3 ml. 12% HCl per gal. milk added slowly with vigorous agitation
0	12	20 sec.	While agitation continues 25·7 g. gluconic lactone powder per gal. milk sprinkled on to the surface of the milk
		30 sec.	Normal quantity rennet added and stirred in
0	13	30 min.	Curd allowed to set
0	43	5 min.	Cut in normal manner (as for Cheddar or Cheshire)
0	48	20 min.	Curd is stirred
1	8	20 min.	Temperature raised at 0·3° F. per min. to 92° F.
1	28	12 min.	Temperature raised at 0·5° F. per min. to 98° F.
1	40	10 min.	Curd allowed to settle
1	50	5 min.	Whey drawn off
1	55	75 min.	Curd cut, piled and drained in usual way
3	10	—	Milled and pressed as usual but with addition of a mixture of gluconic lactone and salt in place of the usual salt alone. Amount of lactone: 11·4 g. to the curd from each gallon of milk

It had initially seemed probable that the rate of acidification during the 'make' would have an important effect on the physical properties of the curd. The experience obtained from Exps. 1–13 supported this hypothesis. Accordingly an attempt was made to adjust the pH with hydrochloric acid at all stages to values similar to those obtaining in the

Cheddar process (Exp. 14). A control cheese was made by the normal process to allow comparative observations to be made throughout. It was noted that the curds differed from each other from the very beginning of setting. Pools of whey appeared on the surface of the 'non-starter' curd, and it was soft, weak in body and readily parted from the sides of the vat. The conclusion was reached that not only was the rate of acidification important but that the acidification must take place within the curd particle. It was here that gluconic acid lactone would be most useful.

The rate of hydrolysis of gluconic acid lactone in milk had already been determined (Fig. 1). From this it was calculated that a reasonable approach to normal conditions of acidity in the curd would be made by an initial acidification of the cheese-milk with hydrochloric acid to a pH of about 6·4 followed just before renneting by enough gluconic acid lactone to give a drop of 0·4 pH unit in the curd during the setting period. In Exp. 15 an acidification within the curd particles was attained by such a process. The resulting curd was normal; it had a good body, did not shrink from the sides of the vat and had no whey on the surface. The difficulty of acidifying the curd during the scalding still remained. In this experiment 2 N-HCl (170 ml.) was added at about 2·5 ml./min. during the scalding period; although this was effective in reducing the pH of the whey the pH of the curd particles remained high.

As the value of acidification during the scalding was doubtful it was omitted in Exp. 16. The salted curd was, however, treated as usual with gluconic acid lactone before pressing. When a sample taken just before pressing was held at room temperature overnight its pH fell to 5·18. This is to be compared with the much slower rate of acidification if lactone is omitted at the salting stage; samples of curd which were not treated with lactone remained at about pH 5·8 for 48 hr. After this time the natural flora from the raw milk brought about a significant fall in pH. It would, therefore, appear that any lack of control over the microflora could be attributed partly to the relatively high pH's existing in the curd between cutting and pressing.

In Exp. 17 the effects on the curd of incorporating various amounts of hydrochloric acid and lactone in the cheese-milk were measured by bowl readings (4). Increasing the lactone to 360 g. still allowed the time of cutting to be selected without difficulty at a normal bowl reading of about 65 (Fig. 2) and had the advantage of producing a lower pH in the curd at pitching. Consequently most of the subsequent experiments employed 360 g. lactone and 20 ml. HCl in 14 gal. milk.

Quality of the ripened cheese. The textures and flavours of the finished cheeses are described in Table 4. Table 5 shows the results obtained when a small group of independent observers were asked to taste selected products. Since most of the cheeses were made according to Cheshire practice, the crumbly texture is normal.

Development of flavour in non-starter cheese. It was soon apparent that in those cheeses which were acceptable the flavour was, without exception, very mild and it did not persist in the mouth like the normal flavour. Various methods of improving the flavour were therefore tried. They consisted mainly in an attempt to encourage the development of lactobacilli in the cheese.

Initially (Exp. 18) raw cheese-milk was inoculated with a strain of *Lactobacillus casei* whose fate could be followed serologically during the cheese ripening (5). Subsequently (Exp. 19) pasteurized milk was used and the size of the inoculum reduced. Various additions were also made to the cheese-milk with a view to improving the curd as a growth

medium for lactobacilli (Exps. 20–22). Later *L. brevis* was also included in the inoculum (Exps. 23–24).

The maximum number of *L. casei* which was found in the ripening cheese was about 10^8/g. and appeared to be independent of the size of the inoculum; the maximum was

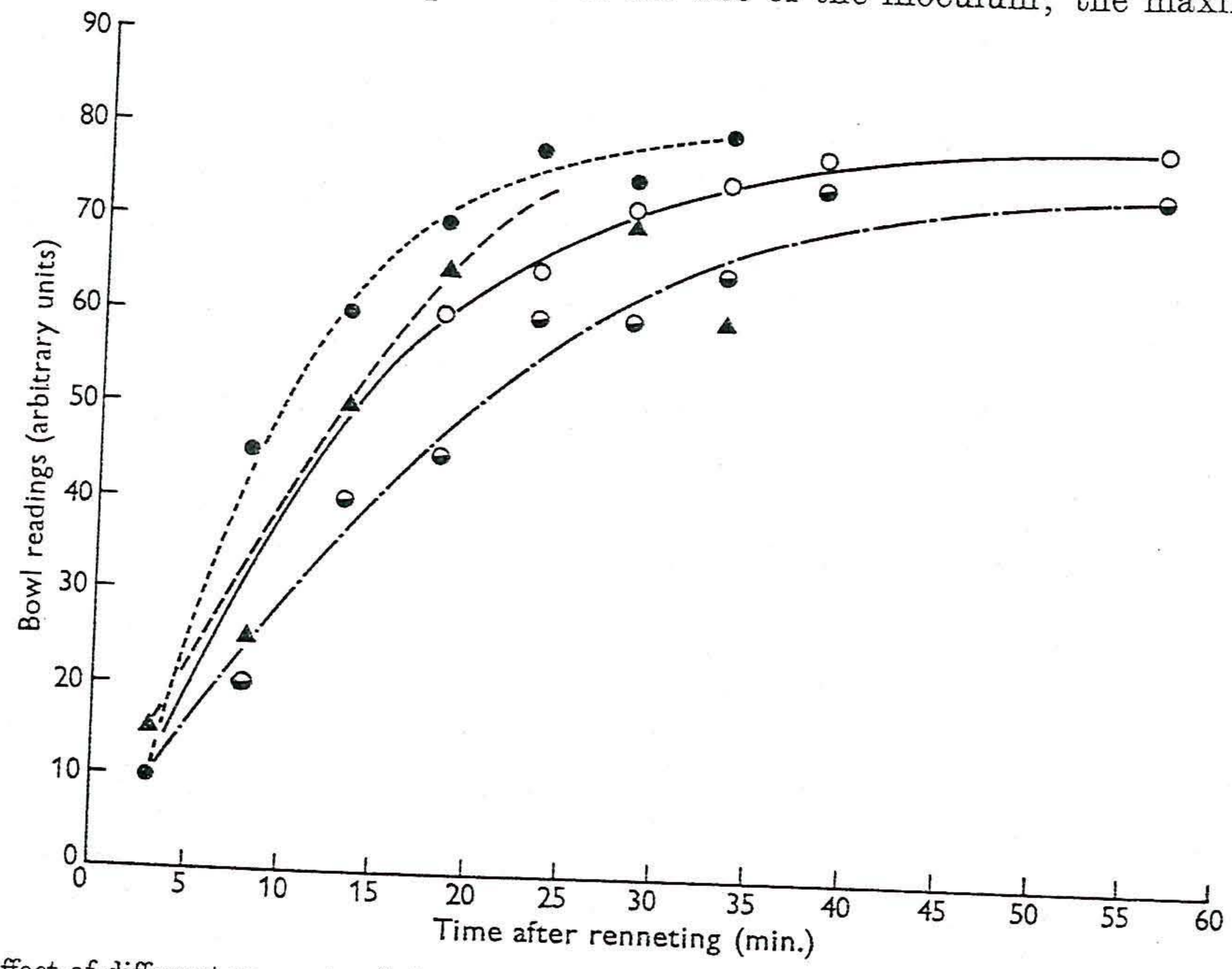

Fig. 2. Effect of different amounts of gluconolactone and/or hydrochloric acid on the consistency of cheese curd as measured by the bowl method. In 14 gal. milk:—◒ 25 ml. 1 0N HCl; ○, 17·5 ml. HCl, 165 g. lactone ● 20 ml. HCl, 360 g. lactone; ▲, 360 g. lactone.

Table 4. *Flavour and texture of cheese made without starter*

Exp. no.	Age of cheese (months)	Texture	Flavour
1	12	Good	Slightly acid, slightly cheesy
2	6	Crumbly	Beery, slightly cheesy
3	5	Blown	Slightly acid, fruity, beery
4	5	Dry, crumbly	Very mild, pleasant, transitory
5	3	Blown, soft, crumbly	Beery
6	3	Blown, soft, crumbly	Beery
7	5	Crumbly	Slightly acid, pleasant
8	3	—	Acid, pleasant
9	5	Crumbly	Slightly acid
10	8	Very good	Slightly cheesy, like 7
11	8	Crumbly	Very acid, fruity
12	7	Crumbly	Rancid, slightly cheesy
13	5	—	Acid, bitter
14	8	—	Potato aroma, slight putrefaction
15	6	Slightly crumbly, satisfactory	Mild, slightly bitter, like processed cheese
16	6	Some gas holes	Very acid, fermented
17	5	Good	Nutty, slightly cheesy
18	6	Hard	Acid, fruity
19	5	—	Very acid, slightly bready, unpleasant
20	$4\frac{1}{2}$	Slight blowing	Vinegary, fruity
21	4	Hard	Acid, very fruity
22	6	—	Acid, fruity, pleasant
23	6	—	Acid, fruity, pleasant
24	4	Crumbly	Acid, pleasant

Table 5. *Summary of results of tasting of non-starter cheese by a group of disinterested observers*

	Do you like it?				Do you prefer it to?–			
	Raw		Cooked		Ration cheese		Processed cheese	
Cheese no.	+	–	+	–	+	–	+	–
4	9	3	4	4	7	4	7	5
7	7(2)	1	5	1	5(2)	3	7(1)	2
9	7(2)	4	5(2)	2	4	10	9	5
10	7(1)	5	5(2)	4	2	8	5	4
15	3(2)	3	2(2)	2	1	7	6	2

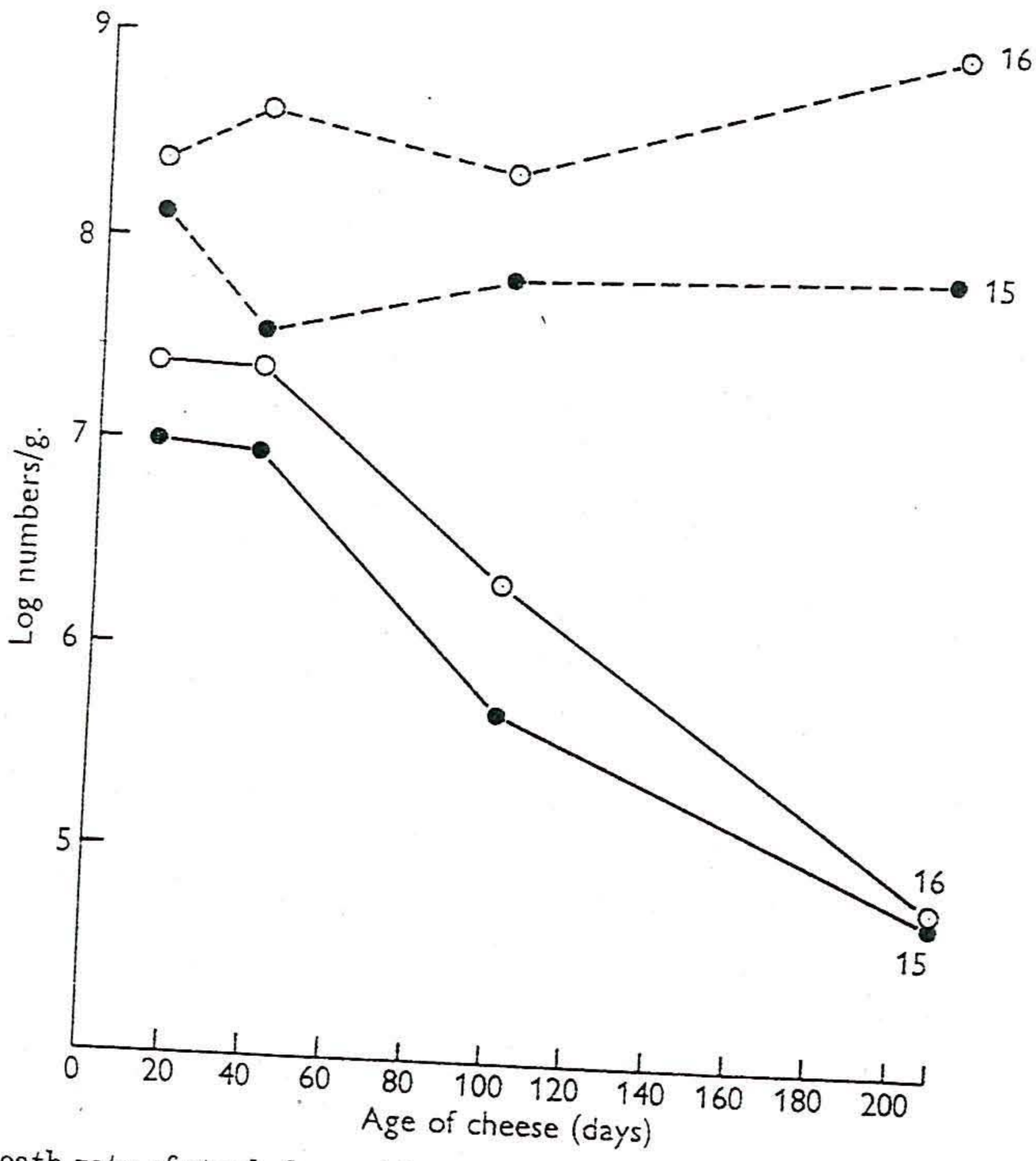

Fig. 3. Death rate of staphylococci in cheeses No. 15 and 16 made without starter.
– – – – Total numbers of organisms; —— Numbers of staphylococci.

more quickly attained when the inoculum was high. *L. brevis*, although growing at the same rate as *L. casei* in the first 2 weeks failed to reach such high numbers.

When the samples taken for bacteriological analysis were tasted, a mature Cheddar flavour was occasionally noted in the early ripening period, e.g. Exps. 18 (3 weeks), 19 (2 weeks), 21 (4 weeks), 34 (6 weeks). The flavour appeared to be transient and could not be found in later samples from the same cheese. No permanent improvement in flavour appeared to be gained, therefore, by inoculating with these strains of lactobacilli.

Development of undesirable organisms in non-starter cheese. The omission of starter inoculum and the relatively high pH of the curd between cutting and milling removes much of the normal control over organisms such as coliforms and staphylococci. For instance, in cheeses 15 and 16, which had been made with raw milk, staphylococci were found in large numbers after 2 weeks of ripening, but they gradually died out (Fig. 3).

The trouble may have been associated with the high numbers of staphylococci in the milk being used at that time. These organisms have not been found in large numbers in non-starter cheese made from pasteurized milk.

Little trouble has been experienced from coliform organisms. Cheeses 18–24 were examined periodically. In the case of the raw-milk cheese 18 the coliforms decreased from 100,000 g.$^{-1}$ at 3 days to 1000 g.$^{-1}$ at 6 weeks, and in the pasteurized milk cheese they exceeded 100 g.$^{-1}$ only on one occasion.

DISCUSSION

A continuous, slowly developing acidity within the curd during the setting stage of Cheddar cheesemaking appears to be essential for good curd texture. Thus the use of gluconic acid lactone, which hydrolyses slowly to produce this acidity, overcame the initial problem of producing a suitable curd without the use of a starter inoculum.

It is improbable, however, that an important stage in a biological process can be replaced by a non-biological system without modifying the resultant products. The substitution of an acid lactone for the starter organisms in cheesemaking modifies the process in two main ways:

(*a*) The hydrolysis of the lactone and hence the production of acid takes place at a maximum rate immediately the lactone is added, i.e. at renneting, and decreases asymptotically. During scalding therefore little acid is being produced within the curd particles when starter is absent; this constitutes a major difference between the gluconic lactone and starter processes, because in the latter the starter organisms reach a peak acid production which is maintained during the setting of the curd and for some time after cutting.

(*b*) The absence of starter activity in cheese made with lactone results in a curd containing a higher lactose content than normal. This would be expected to influence the microflora developing during the ripening period. The effect of washing the curd has not yet been explored.

No simple method for the satisfactory acidification of the curd during the scalding process has yet been found. Consequently the growth of coliforms and other undesirable organisms might occur in this period in the absence of any control due to acidity or to the competition from starter bacteria. A detailed study of the flora of cheese made with gluconic acid lactone still remains to be done. In a few of the cheeses which were examined, multiplication of staphylococci was noted, but the resultant cheeses were safe to eat. No development of undesirable flora has been noted in cheese made from pasteurized milk.

The cheeses made without starter had generally two properties in common; the texture was crumbly and the flavour acid. No Cheddar flavour developed in any of the cheese, but it was very noticeable that the flavour improved after cooking or toasting. Inoculations with *L. casei* or a mixture of *L. casei* plus *L. brevis* failed to produce a Cheddar flavour, although the organisms grew satisfactorily. It could be inferred that the products of the starter are important in the production of good flavour, but it has also to be remembered that the lactobacilli inoculated into these cheeses were probably utilizing lactose as a carbon source. The carbon source of lactobacilli in normal Cheddar cheese is not known, but no lactose is present at the time when they are developing vigorously. Thus

the products of their fermentation in normal cheese may be quite different from those formed in the 'non-starter' cheese.

The experiments which have been described were carried out mainly to discover whether methods of artificial acidification were worth exploring. For this reason no elaborate measurements of the properties of the curd or of the cheese were undertaken; an attempt was made to produce curd which appeared normal to the observer. In this success was achieved and it is clear that the way lies open for the prosecution of further research with the aid of more refined techniques. It is hoped that the absence of starter organisms will permit a closer study both of the behaviour of curd in the vat and of the effect of various added micro-organisms and enzymes on the ripening of cheese.

Further experiments are necessary before it can be decided whether the obvious advantages of speed and regularity are worth the cost of the materials; moreover it is not yet known whether the flavour of such cheeses would be generally acceptable. On the other hand, it is quite likely that occasional starter failure could be successfully countered by the use of gluconic lactone.

SUMMARY

Cheese has been successfully made from raw and pasteurized milk by a process similar to that employed for Cheddar or Cheshire-type cheese but omitting the starter inoculum.

In the preferred method gluconic acid lactone was used as the acidifying agent during the setting of the curd and after milling.

The flavour of the best products was acceptable to a number of people not necessarily experienced in cheese-tasting, but it was different from that of normal Cheddar.

No improvement in flavour was achieved by inoculation of the cheese milk with a strain of *L. casei* and *L. brevis*.

Further detailed investigation is needed to perfect the process and to determine whether its use in industry is justified or desirable.

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