INHIBITION OF GROWTH OF BUTTER CULTURES IN NATURALLY RIPENED CREAM AFTER NEUTRALIZATION

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SUMMARY

Growth of *Streptococcus diacetilactis* cultures was slower and diacetyl production lower in bulk factory cream after neutralization than in low-acid cream.

Growth and diacetyl production in some individual samples of ripened cream after neutralization was similar to that in low-acid cream, but in other samples growth was inhibited. Approximately 50 per cent. of the samples of high-acid cream as received at the butter factory were inhibitory to the growth of the starter culture.

This inhibition was found to be due to the presence of inhibitory streptococci, which were strains of *S. lactis*. Such inhibitory organisms were also found in fairly large numbers in creams which were not inhibitory.

When acid production took place normally in a ripened cream, the rate and amount of diacetyl production were the same as in a low-acid cream.

I. INTRODUCTION

In most countries cream to be used for cultured butter manufacture is selected from supplies of choice cream with a low initial acidity. Under Queensland conditions most cream for buttermaking has been naturally ripened on the farm, and the bulk choice cream at the factory frequently has a titratable acidity between 0·20 and 0·40 per cent. The results of early experiments in Queensland on the culturing of cream for buttermaking were reported by Smythe and Crittall (1959). In these investigations the acidity of the raw cream before neutralization was usually between 0·30 and 0·40 per cent.

Following this work, investigations were made on a laboratory scale to obtain information concerning the effect of different conditions of incubation on the growth of butter cultures and diacetyl production. The results obtained using low-acid choice cream have been published (Lightbody 1962). In the course of these experiments it was noted that only poor growth of the cultures occurred in bulk factory cream and some samples of high-acid creams. The experiments reported here were made to investigate this and to determine the incidence of inhibitory cream supplies.

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II. METHODS

A strain of *Streptococcus diacetilactis,* DRC2, was used in all experiments.

Before inoculation of the cream samples, the fat concentration was reduced to 35 per cent. by the addition of skim-milk, the cream was neutralized to a pH of 6·8–7·0 by the addition of N NaOH, and then pasteurized by batch heating to 80°C. The inoculation rate was maintained at 1 per cent. and incubation was at 16°C.

Determinations of pH, titratable acidity and diacetyl were made as in previous experiments (Lightbody 1962).

Raw cream samples were plated on bromcresolpurple milk agar to determine acid-producing organisms. This medium (BCP milk agar) contained 1·5 per cent. agar, BCP indicator and 0·2 per cent. $CaCO_3$ in suspension. The volume of sterile skim-milk added to the plate was 1 ml. On this medium organisms capable of producing acid from milk without the presence of glucose or yeast extract developed yellow colonies with a yellow halo. The chalk suspension prevented acid from one colony spreading over a wide area of the plate.

Some samples of cream were plated on layer plates to detect the presence of inhibitory organisms, using a modification of the method of Kelner (1948). "Difco" tryptone-glucose-yeast extract agar (TGE) was used for all layers. A basal layer of 10 ml was poured, the inoculum mixed with 5 ml of agar, then a buffer layer of 10 ml added. After 24 hours' incubation, a lawn of DRC2 was added by inoculating 10 ml agar with 0·5 ml of a 1 in 10 dilution of a fresh 24 hr culture. Well-marked zones of inhibition were formed around the inhibitory colonies, which could be picked off the plates easily.

Colonies isolated from plates were examined for production of inhibitory substances by the 50 per cent. whey test used by Lightbody and Meanwell (1955).

III. RESULTS

(a) Growth of DRC2 in Different Cream Samples

In all cases in which starter growth and diacetyl production in bulk choice cream were compared with growth in low-acid cream, there was evidence of some inhibition of growth in the bulk cream. This inhibition was not apparent in several individual farm samples of high-acid cream.

The results of one experiment in which growth was compared in three creams —a low-acid cream from one supplier, a high-acid cream from one supplier, and bulk choice cream with high acidity—are shown in Figure 1. In this experiment, growth in the low-acid cream was slightly faster than in the neutralized high-acid cream, but in both creams high levels of diacetyl were produced. No definite peak

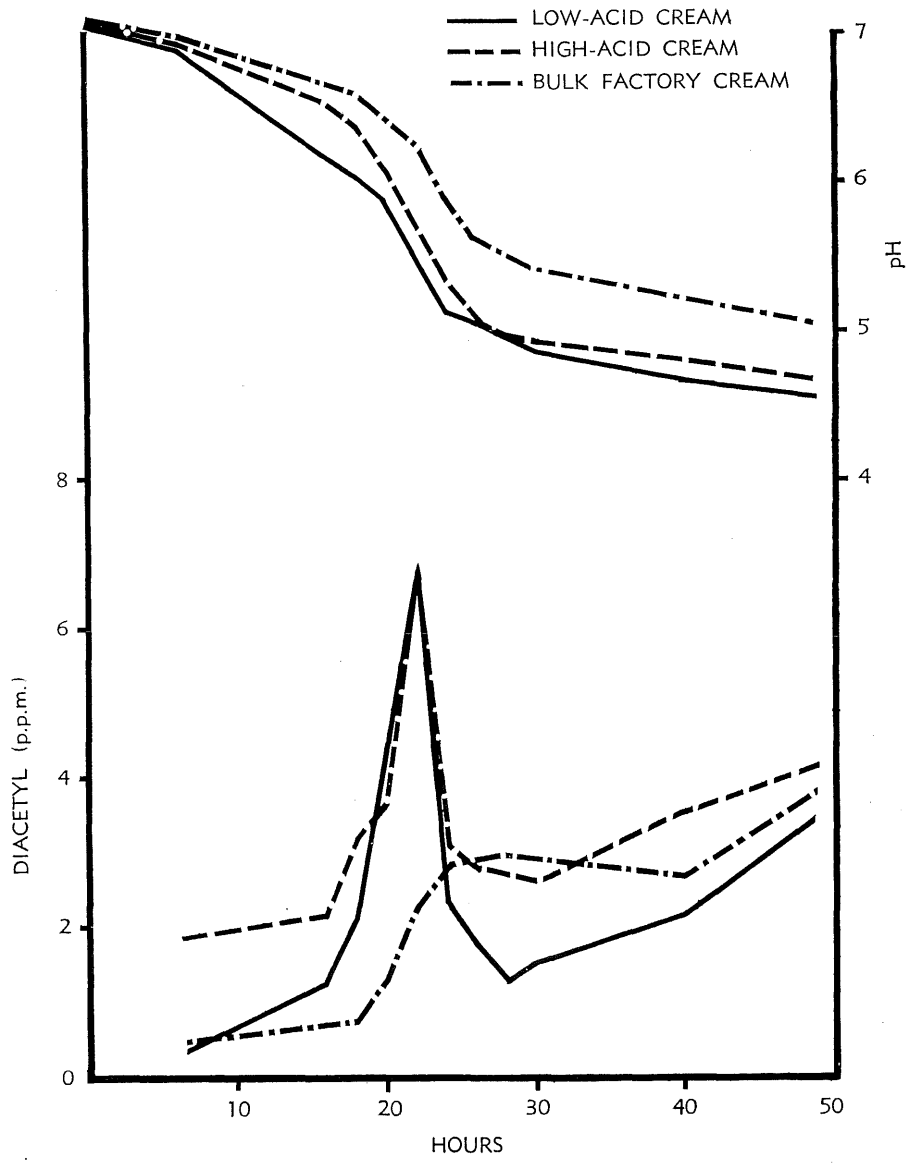

Fig. 1.—Starter growth and diacetyl production in three cream samples.

in diacetyl production occurred in the bulk cream, and culture growth was considerably slower than in either of the other two creams. In some similar experiments, a peak of diacetyl concentration was obtained in the bulk cream, but the amount present at this time was much less than in the other creams.

(b) Growth of DRC2 in Cream Before and After Natural Ripening

Cream samples were obtained with low initial acidity and allowed to ripen in the laboratory. Subsamples were taken before and after ripening and tested for their ability to support the growth of DRC2 after neutralization and pasteurization. In some experiments estimations for pH, acidity and diacetyl were made at 2 hr intervals from 16 to 30 hr and in others after 20 and 24 hr. The results of analyses after 20 and 24 hours' incubation of three of these creams are shown in Table 1.

TABLE 1

EFFECT OF RIPENING ON SUBSEQUENT GROWTH AND DIACETYL PRODUCTION IN NEUTRALIZED, PASTEURIZED CREAM

No.	Initial		pH after Neutralization	After 20 hr Incubation		After 24 hr Incubation	
	pH	Acidity		pH	Diacetyl (p.p.m.)	pH	Diacetyl (p.p.m.)
1 ..	6·7	0·10	6·6	5·4	6·8	5·2	4·3
	4·4	0·39	6·9	6·8	0·4	6·8	0·4
2 ..	6·7	0·09	6·8	6·3	0·6	6·0	1·2
	5·5	0·25	6·9	6·8	0·1	6·8	0·1
3 ..	6·8	0·11	6·5	5·9	2·6	5·7	4·4
	4·7	0·30	6·7	6·2	1·3	5·9	2·5

In Sample 1, the culture failed to grow in the cream when it was ripened to a pH of 4.4 before neutralization and pasteurization. Sample 2 was ripened to an acidity of 0·25 per cent. and pH 5·5. Again the culture failed to grow in this cream after treatment. With Sample 3, ripening prior to neutralization and pasteurization slightly retarded the rate of acid production, but comparable amounts of diacetyl were produced at the various pH levels.

The above experiments showed that there was considerable variation among individual cream samples. Some samples of acid cream completely inhibited growth of the culture, whereas with other acid creams starter growth after neutralization and pasteurization was comparable with that in the low-acid cream.

(c) Incidence of Inhibitory Properties in Cream Supplies

Samples of choice cream were taken on receival at a butter factory and examined for their ability to support growth of DRC2 after neutralization and pasteurization. Determinations of pH, acidity and diacetyl were made after 20 and 24 hours' incubation at 16°C.

Some creams caused marked inhibition of culture growth, as shown by the reduction in the amount of acid produced. A summary of these results is given in Table 2. Almost 50 per cent. of the acid creams caused marked inhibition of starter growth. Samples of cream from some farms were taken on more than

one occasion. In the majority of cases a farm supplying cream containing inhibitory substances on one occasion again supplied inhibitory cream at the second sampling some months later. No further investigation was made of the three samples of low-acid cream which showed inhibition, but this inhibition was possibly due to the presence of antibiotics used for mastitis treatment.

TABLE 2

GROWTH OF DRC2 IN CHOICE CREAM FROM INDIVIDUAL SUPPLIERS AS RECEIVED AT A BUTTER FACTORY

Type of Cream	No. of Suppliers	No. of Samples	No. Showing Inhibition
High-acid (pH <5)	64	96	47
Slightly acid (pH 5–6·3) ..	13	13	2
Low-acid (pH >6·3)	31	33	3
Total		142	52

Diacetyl estimations were made on the creams after 20 and 24 hr. The amount of diacetyl produced was generally related to the pH at the time of testing. No attempt was made to compare diacetyl production in the individual creams. Because of the very rapid change in diacetyl content at pH values between 6 and 5, this could not be done when only two measurements were made.

Examples of inhibitory cream were plated on BCP milk agar. Representative acid-producing organisms, which were the dominant type on the plate, were picked off and purified. The cultures were then examined for inhibition by the 50 per cent. whey test (Lightbody and Meanwell 1955). Most creams examined yielded some inhibitory organisms when 30–50 colonies were picked from the plates. The inhibitory cultures were further examined by morphological and biochemical tests, and found to be typical cultures of *Streptococcus lactis*.

Some of the cream samples were also plated on TGE layer plates to detect inhibitory colonies. All of the inhibitory creams showed inhibitory colonies by this method. Although only a small percentage of the organisms present in the cream were inhibitory, the numbers of such organisms were greater than 10^7. Some creams which did not appear to be inhibitory to the growth of DRC2 also showed some inhibitory colonies at a dilution of 1 in 10^6.

The inhibitory cultures isolated from the creams were examined for inhibition against strains of *S. lactis, S. cremoris* and other strains of *S. diacetilactis*. They were found to inhibit all cultures tested.

IV. DISCUSSION

In Queensland, very little low-acid cream is received at butter factories for many months each year, so that cream for culturing has been selected from bulk choice cream after neutralization. Starter growth in such cream was usually

rather slow. This inhibition of growth was not found in all high-acid creams, but was due to the presence in some creams of inhibitory streptococci. These were found to be strains of *S. lactis,* and were presumably nisin-producing organisms. The incidence of these inhibitory streptococci in the acid creams was high, as approximately 50 per cent. of the supplies were inhibitory to the growth of starter cultures. Other cream supplies, in which inhibition was not noticed, also contained fairly large numbers of inhibitory organisms.

The low-acid creams were practically all collected in July and August, when air temperatures were low. At these times the pH of the bulk cream was fairly high, and the cream supported good starter growth. In the hotter months, most of the cream reaching the factory had high acidity. Although starter growth was completely inhibited in some individual cream samples, the amount of inhibitory substances in the bulk cream merely retarded starter growth. Diacetyl production was linked to starter growth. Provided growth took place normally there was no reduction in diacetyl production. When diacetyl is to be produced by starter growth in the cream it would appear necessary to select, as far as possible, cream supplies with low acidity. All starter cultures tested were susceptible to the inhibitory organisms.

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