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RIPENING OF RAW FARM CREAM IN QUEENSLAND

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SUMMARY

Cream samples taken immediately after separation at the farm were examined before and after storage at 4·5, 10, 15·5 or 21°C for bacteriological quality, pH, chemical changes and grading.

The growth rates of various groups of organisms were measured at each temperature. Spoilage organisms and lactic streptococci increased in count at similar rates in most samples, and the growth of the spoilage organisms was not inhibited until the pH was lowered. Most of the organisms causing defects in cream were Gram-negative rods, many of which were psychrophilic.

Creams which did not contain large numbers of spoilage organisms at or before the time of degrading were degraded due to fat breakdown at low pH. The addition of an acid-producing organism to suppress the growth of Gram-negative rods did not prolong the period before deterioration.

Storage at 10°C produced the most satisfactory grading results. Although bacterial growth was markedly depressed at 5°C, samples were sometimes degraded due to chemical deterioration of the fat. The use of farm refrigeration to reduce cream temperature to 10°C, and the reduction of contamination of cream to a minimum by paying strict attention to hygiene, seem to offer the best method of ensuring choice quality when cream is received at the factory.

I. INTRODUCTION

In Queensland, cream supplies to most butter factories are received only three times a week. Cream must therefore be held on the farm for varying periods up to 3 days. Although some farmers have provided some form of refrigeration for their cream, in many cases cream cannot be cooled much below atmospheric temperature.

As all cream for buttermaking is neutralized before churning, cream with a fairly high acidity but without off-flavours is accepted as of choice quality, and can be made into choice butter. Some farmers with good methods of hygiene have been able to obtain choice cream without refrigeration, while other farmers who are apparently using similar methods frequently have their cream degraded. The studies reported here were therefore undertaken to investigate bacteriological and chemical aspects of the ripening of some farm creams.

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II. METHODS

Cream samples were taken at the farm after the morning milking directly from the separator spout, immediately chilled in iced water, and forwarded to the laboratory. They were received at midday and testing was commenced immediately.

Subsamples of each cream were held at 4·5, 10, 15·5 and 21°C. Each was examined initially and after 1, 2, 3 and 4 days' storage both bacteriologically and chemically. Total bacterial counts were made on tryptone-glucose-yeast extract (TGE) agar (Difco), and counts of acid-producing organisms and proteolytic organisms were made on BCP-chalk-milk-agar (Lightbody 1962), with incubation for 48 hr at 32°C. The only types of bacteria which were found to produce acid on BCP-chalk-milk-agar within 48 hr, apart from the lactic streptococci, were coliform organisms. Counts of coliform organisms were made on desoxycholate agar (Difco) with incubation at 32° for 24 hr. Lipolytic organisms were estimated on victoria blue fat agar (Jones and Richards 1952) with incubation for 5 days at 32°C, and psychrophilic organisms were counted on TGE agar after incubation for 14 days at 5°C. Organisms able to hydrolyse tributyrin were counted on double-layer plates, the basal layer being nutrient agar and the thin upper layer of 5 ml of agar containing the inoculum and 0·5% tributyrin. Incubation of tributyrin agar plates was for 5 days at 32°C. Counts of Gram-negative rods were made on TGE agar containing 2·5 i.u. penicillin per ml (Lightbody 1964).

The creams were also examined for pH, titratable acidity and grade. In some cases estimations were made for fatty acid values on fat obtained by extraction (Johnson and Gould 1949), for lipase activity by a modification of the method of Stadhouders and Mulder (1958), and for peroxide values (Loftus Hills and Thiel 1946).

Samples were obtained from 33 suppliers who frequently had cream degraded at the factory and from 16 suppliers with a consistently good record of grading. Cream was not refrigerated on any of these farms. Visual inspection by the district dairy officer did not reveal any obvious defects in hygiene at any of the farms from which samples were collected.

Organisms isolated were first grouped on the basis of Gram reaction and morphology. Gram-negative rods were further classified by the methods used by Lightbody and Petersen (1962). Cultures producing a red water-insoluble pigment were considered to be *Serratia.* In other aspects these organisms agreed with the description given by Skerman (1959). Gram-positive cocci were classified according to the schemes given by Abd-el-Malek and Gibson (1948*a,* 1948*b*). Gram-positive sporing rods were considered to belong to the genus *Bacillus.* Gram-positive non-sporing rods, similar to the description given by Abd-el-Malek and Gibson (1952), were considered to be *Corynebacterium.*

III. RESULTS

(a) Initial Quality

The initial bacteriological quality of the cream showed no relationship to the history of grading results (Table 1) and several creams in both groups had high bacterial counts. For this table the highest of the counts for psychrophilic, lipolytic, proteolytic or coliform organisms was taken as the number of spoilage organisms. Subsequent examination of the effect of temperature of storage and type of bacterial flora on bacterial counts and grading of the cream was made without consideration of the previous history of grading results for the various farms.

TABLE 1

SUMMARY OF INITIAL BACTERIOLOGICAL QUALITY OF CREAM SAMPLES

—	From Farms with Good Grading Results	From Farms with Poor Grading Results
No. of samples	16	33
Total count per ml—		
Mean	5·50±0·64	5·68±1·04
Range	4·3 —6·8	3·8 —6·9
Acid-producing organisms per ml—		
Mean	4·07±1·42	4·32±3·02
Range	2·0 —6·6	1·0 —6·9
Spoilage organisms per ml—		
Mean	4·40±0·75	4·04±1·27
Range	3·2 —6·4	1·0 —6·3

Bacterial counts in logarithms

(b) Effect of Storage Temperature on Bacterial Growth

Most cream samples stored at 4·5° showed little change in bacterial counts during the 4-day storage period. A few creams showed a considerable increase in the psychrophilic count, but only occasionally did the numbers of psychrophiles become sufficiently high to increase the total count and only in one sample was there sufficient bacterial growth to be likely to influence grading.

Bacterial growth was fairly slow at 10°C (Figure 1). In cream 1A (Figure 1), initial bacterial counts were low, and high counts were not reached until after 4 days' storage. Coliform counts were still less than 10 at the end of the storage period. There was very little change in pH. Cream 1B had considerable contamination initially and very high counts were reached by the third day. In both cases there was a tendency for the count of psychrophiles to increase more rapidly than the other counts, as was the case in practically all creams stored at this temperature.

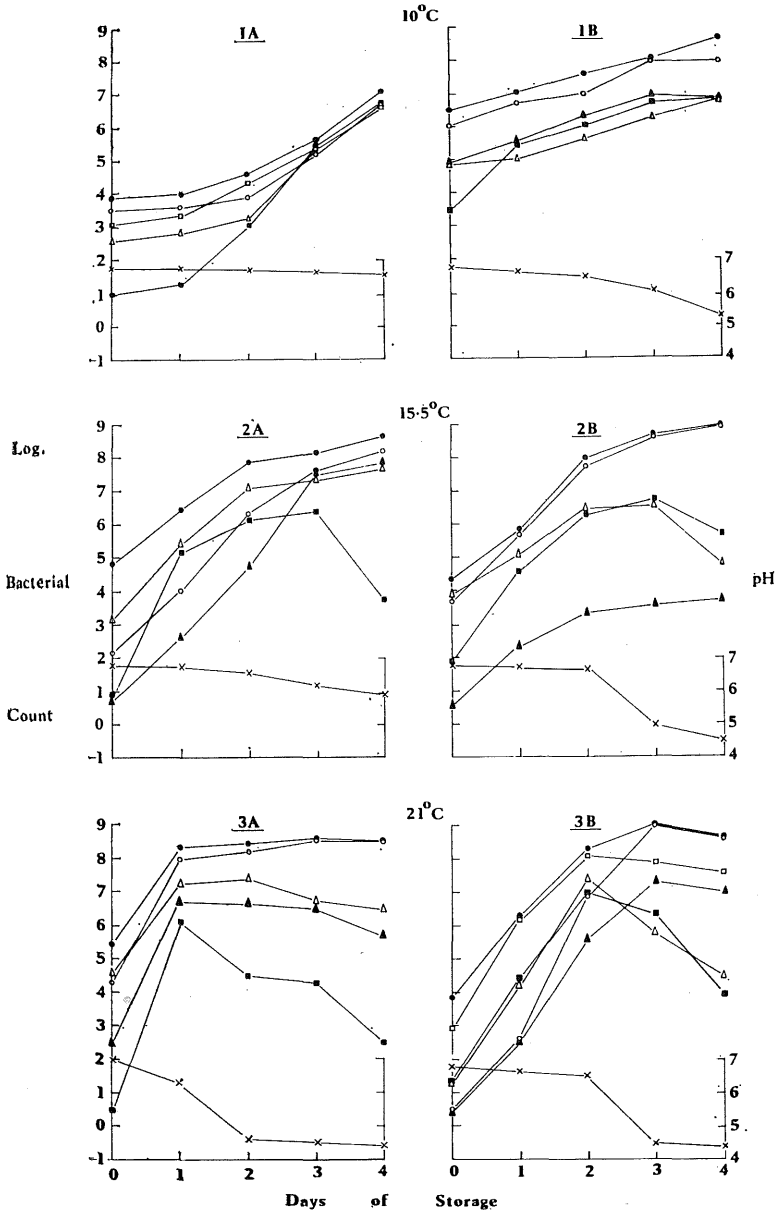

Fig. 1.—Changes in bacterial counts and pH of 6 cream samples during storage for 4 days at 10, 15·5 or 21°C.

The growth rates of various types of bacteria at 15·5°C are shown in Figure 1. In cream 2A, coliforms and acid-producing organisms grew at approximately similar rates. The growth rate of psychrophiles appeared faster initially but slowed markedly as the number of acid-producing organisms reached a maximum. The number of casein-digesting organisms was quite high in this

cream and these grew at a rate comparable with that of acid-producing organisms for the first 2 days. In cream 2B, the count of casein-digesting organisms did not increase as rapidly as in 2A, and after 2 days' storage the organisms present were predominantly acid-producing organisms. The psychrophilic count increased rapidly until growth of these types of organisms was suppressed when the pH began to fall.

Figure 1 shows that in cream 3A at 21°C, psychrophiles, coliforms, acid-producing organisms and casein-digesters all grew quickly during the first day, until the acid-producing organisms approached a maximum count. Cream 3B was produced under good conditions with a total count initially of 6,000 per ml and numbers of psychrophiles, coliforms and casein-digesters very low. Growth of all groups of organisms was rapid and quite high levels were reached after 2 days.

(c) Importance of Various Bacterial Types

Colonies were selected from high dilution plates ($1/10^6$ or greater) of the first 32 creams examined. After purification, the cultures were tested for their ability to produce defects in heat-treated cream. When more than one isolate from any cream sample gave similar reactions only one was retained for detailed study. This method of selection of colonies cannot be used to give any indication of the relative importance in cream deterioration of the various groups of organisms isolated but it does show the types of spoilage organisms frequently encountered.

The organisms were classified into the following genera, with the number of isolates given in brackets: *Pseudomonas* (27), *Escherichia* and *Aerobacter* (30), *Vibrio* (16), *Achromobacter* (7), *Micrococcus* (17), *Corynebacterium* (8), *Serratia* (2), *Bacillus* (2), *Alkaligenes* (1). Eighteen strains of streptococci were also examined, of which 4 were malty strains of *Streptococcus lactis,* 8 were *S. liquifaciens,* 5 were *S. faecalis,* and 1 was *S. thermophilis.* The streptococci produced bitterness or other off-flavours.

Putrefactive, bitter or fruity flavours were usually produced by the Gram-negative rods, but some strains of micrococci and corynebacteria also produced marked bitterness and off-flavours. Most of the organisms producing defects were strongly proteolytic and/or attacked tributyrin. However, many organisms which hydrolysed tributyrin, including many Gram-negative rods, did not give any evidence of lipolysis on victoria blue fat agar.

(d) Categories According to Bacterial Flora and Development

For convenience, the creams have been grouped into four categories depending on the type and behaviour of the bacterial flora during storage at 15·5 and 21°C:

(i) Creams in which the initial count was high but practically all organisms were acid-producers.

(ii) Creams in which the numbers of spoilage organisms were low initially and the numbers of acid-producing organisms were 10 to 100 times greater. In such creams the growth of spoilage organisms was suppressed when high numbers of acid-producers had developed.

(iii) Creams in which the numbers of spoilage organisms and acid-producers were both low initially, so that spoilage organisms were present in large numbers at the time the cream became acid. It was often not possible to tell whether or not these spoilage organisms contributed to degrading.

(iv) Creams with fairly high bacterial counts initially, with both acid-producers and spoilage organisms present in large numbers.

Of the 49 creams examined, 10 were allotted to group 1, 13 to group 2, 20 to group 3, and 6 to group 4. Most of the creams in groups 1 and 2 were degraded when the pH became low without the occurrence of large numbers of spoilage organisms, while some of the creams in group 3 and all of the creams in group 4 were degraded following the development of large numbers of spoilage organisms which probably contributed to the degrading.

(e) Grading of Creams

The type of deterioration as judged by grading comments appeared to be similar at 15·5 and 21°C, but the cream usually remained choice for one day longer at the lower temperature. Most creams were degraded as soon as they became acid. Only 4 of 40 creams stored at 21° and 9 of 49 creams stored at 15·5°C had a pH over 5·5 at the time of degrading, and in only one sample (stored at 15·5°C) were there fewer than 10^7 acid-producing organisms per ml at degrading. On the other hand, only 14 creams were still choice after the pH became lower than 5·0.

The bacteriological results were inspected to determine whether spoilage organisms were present at or before degrading in sufficient numbers to cause or contribute to the degrading. These results for 49 creams stored at 15·5°C are shown in Table 2. Most creams in groups 1 and 2 had relatively low counts of spoilage organisms and all creams in groups 3 and 4 had high counts of these organisms. The relative importance of coliforms and psychrophiles among the spoilage organisms is also shown in this table.

TABLE 2

DISTRIBUTION OF HIGH COUNTS OF SPOILAGE ORGANISMS IN CREAM SAMPLES STORED AT 15·5°C, AT THE TIME OF OR BEFORE DEGRADING

Group	No. of Samples	No. with Spoilage Organisms			No. with High Counts of		
		$< 10^6$	10^6–10^7	$> 10^7$	Coli-forms	Psychro-philes	Both
1	10	4	4	2	..	1	1
2	13	3	6	4	..	2	..
3	20	..	..	20	6	4	10
4	6	..	..	6	2	2	2
Total	49	7	10	32	8	9	13

Table 3 shows similar results for creams stored at 21°C. There were proportionately more samples without high numbers of spoilage organisms at or before degrading at 21° than at 15·5°C. The relative importance of psychrophilic organisms among the spoilage types was somewhat less at the higher temperature.

TABLE 3

DISTRIBUTION OF HIGH COUNTS OF SPOILAGE ORGANISMS IN CREAM SAMPLES STORED AT 21°C, AT THE TIME OF OR BEFORE DEGRADING

Group	No. of Samples	No. with Spoilage Organisms			No. with High Counts of		
		$< 10^6$	10^6–10^7	$> 10^7$	Coliforms	Psychrophiles	Both
1	7	4	..	3	1	1	..
2	12	7	3	2	2	..	..
3	16	1	3	12	3	3	4
4	5	..	1	4	4	..	..
Total	40	12	7	21	10	4	4

Grading comments on the acid creams not containing large numbers of spoilage organisms were usually "greasy," "tallowy" or "overripe and stale," and similar comments were also made on some of the creams in which spoilage organisms were present in large numbers.

The most satisfactory grading results were obtained when the cream samples were stored at 10°C. Eight of 45 samples stored at this temperature were degraded after 3 days. In 6 of these creams this degrading was associated with extremely high counts of spoilage organisms. These creams were badly contaminated from farm equipment as each of them contained more than 10^6 organisms per ml before storage. Two creams stored at 10°C were degraded without the occurrence of large numbers of bacteria and with defects similar to those commonly occurring in creams stored at 4·5°C.

Of 45 samples stored at 4·5°C, 12 samples were degraded after 3 days and off-odours or off-flavours were noted in another 14 samples which were not degraded. Even in the case of creams which did not develop definite off-flavours, the graders frequently commented on a better flavour in the sample stored at 10° than in that stored at 4·5°C. In all creams the number of bacteria was greater after 3 days in the subsample stored at 10° than in that stored at 4·5°C, and the count was not sufficiently high in the cream stored at the lower temperature to cause off-flavour development.

(f) Addition of Acid-producing Organisms to Cream

Four experiments were made in which 1% of a mixture of three strains of *S. lactis* in skim-milk was added to cream before storage at 21°C. The cultures selected had been isolated from cream samples, were non-malty, were not capable of hydrolysing tributyrin and had moderate rates of acid production. The results

of one such experiment are shown in Table 4. Growth rates of spoilage organisms were markedly suppressed. However, the cream to which acid-producing organisms were added was degraded at the same time as or before the control cream.

TABLE 4

EFFECT OF ADDING 1% OF A CULTURE OF *S. lactis* TO CREAM BEFORE STORAGE AT 21°C

—	Control				With Added *S. lactis* Culture			
	Initial	After 1 day	After 2 days	After 3 days	Initial	After 1 day	After 2 days	After 3 days
pH	6·8	6·3	4·9	4·4	6·3	4·6	4·4	4·3
Total count per ml	5·8	8·2	9·2	9·0	7·4	9·2	9·2	9·2
Acid-producing organisms per ml	5·5	7·8	8·4	8·7	7·4	9·2	9·3	8·2
Penicillin-resistant organisms per ml	4·5	7·6	8·4	7·6	4·5	5·2	5·4	3·8
Coliforms per ml	2·2	6·7	7·8	5·4	2·2	3·7	3·0	1·0
Grade	C	C	C	bare C	C	C	1Q	1Q

Bacterial counts in logarithms.

(g) Chemical Changes in Cream Subsamples Stored at 4·5°C

Some samples showed an appreciable increase in titratable acidity during low-temperature storage, although the count of acid-producing organisms was low. Estimations for fatty acid values and peroxide values were made on 26 samples before and after storage at 4·5°C. Fatty acid values were in the range of 0·13 to 0·44 before storage and 0·23 to 1·46 after storage. The correlation coefficient between increase in titratable acidity and increase in fatty acid value was 0·7, which was highly significant ($P < 0·001$).

TABLE 5

RELATIONSHIP BETWEEN INCREASED TITRATABLE ACIDITY DURING STORAGE AT 4·5°C FOR 4 DAYS AND GRADING

No. of samples in each grade category

Grading	Increase in Titratable Acidity as % Lactic Acid		
	< 0·04	0·04–0·06	> 0·06
Cream degraded	5	3	7
Off-flavours or off-odours noted ..	2	6	7
Choice—no comments	12	..	3
Total	19	9	17

Increases in titratable acidity were compared with grading comments. The results are shown in Table 5. A chi-square test was carried out on the results, with titratable acidity increases of 0·04% and greater considered in one class. This showed a significant correlation between titratable acidity increase and grading (chi-square = 14·41, $P < 0·001$). Seven samples which received adverse grading comments did not show increased titratable acidity.

The results of tests for fatty acid values and peroxide values in relation to grading for 26 samples are shown in Table 6. Only 2 of 18 samples which received adverse comments did not show fat deterioration indicated by increased peroxide values or increased fatty acid values.

TABLE 6

RELATIONSHIP BETWEEN FATTY ACID VALUES AND PEROXIDE VALUES AND GRADING COMMENTS OF CREAM SAMPLES AFTER STORAGE FOR 4 DAYS AT 4·5°C

No. of samples in each grade category

Grading	PV <0·20 and FAV <0·50	PV 0·20–0·30 or FAV 0·50–0·80	PV >0·30 or FAV >0·80
Degraded	1	..	6
Off-flavours or off-odours ..	1	6	4
No comments	5	1	2

PV — peroxide value
FAV — fatty acid value

Estimations for lipase activity were made on 17 cream samples. There was some correlation between lipase values initially and fatty acid values after storage at 4·5°C ($r = 0·58$, $P < 0·02$).

(h) Chemical Changes in Cream Subsamples Stored at 15·5 and 21°C

Twenty-six samples were examined for fatty acid values and peroxide values after storage at 21°C, and 19 samples after storage at 15·5°C. Fatty acid values were lower for most samples after storage at these temperatures than after storage at 5 or 10°C. There were exceptions in the case of four samples with very high fatty acid values associated with the presence of large numbers of lipolytic organisms.

Peroxide values showed a greater increase following storage at 15·5 or 21°C than following storage at the lower temperatures. There tended to be an inverse relationship between peroxide value and fatty acid value, as shown in Table 7. All but three of these creams were degraded at the time they were tested for peroxide value and fatty acid value. There was no relationship between high peroxide values and grading comments, and several creams degraded because of tallowiness had low peroxide values.

TABLE 7

RELATIONSHIP BETWEEN FATTY ACID VALUES AND PEROXIDE VALUES ON CREAM SUBSAMPLES STORED AT 15·5 OR 21°C FOR 4 DAYS

No. of samples

Peroxide Value	Fatty Acid Value		
	<0·50	0·50–0·80	>0·80
<0·10	3	4	3
0·10—<0·20 ..	6	7	2
0·20 and over ..	15	5	..

IV. DISCUSSION

In these experiments counts of various groups of organisms have been obtained by the use of differential media. Emphasis has been placed on groups likely to cause off-flavours in creams and only a limited amount of work has been done on isolation and classification of the types of bacteria present at any time. The former method has allowed estimates of the population of various groups to be made when the numbers of such groups were small compared with the total bacterial population, whereas the latter method permits identification only of the predominant bacterial types. It has been shown here that even very small numbers of certain species can be important in subsequent deterioration of the product, and when total bacterial counts are high, types of bacteria comprising only a small percentage of the total may be of importance with regard to quality.

It has been fairly generally accepted that if a cream is "cleanly-produced" and there is good growth of lactic streptococci, growth of spoilage organisms, at least Gram-negative types, will be suppressed and a good clean acid cream will result. In these experiments growth of lactic acid bacteria depressed the growth of Gram-negative rods once the pH started to fall, but there was no evidence of any effect on the growth of these organisms before very high counts of lactic acid bacteria were attained. Pont (1935*a*) stated that development of acidity was an important factor in maintaining the quality of raw cream, but in the present experiments acid creams were degraded soon after a low pH was reached, so the suppression of the growth of Gram-negative rods only slightly delayed the degrading. It was also found that many types of Gram-negative rods grew at least as fast as the acid-producing organisms at 15·5 or 21°C, so that by the time the pH started to fall there were large numbers of these spoilage organisms present.

A bacterial count of at least 10^7 per ml appeared to be necessary before off-flavour defects caused degrading. Similar levels were quoted by Punch, Olson, and Thomas (1961) for various types of psychrophilic organisms at the time of detection of a flavour change in milk. Types of organisms causing defects when

inoculated into cream were predominantly Gram-negative rods. Although coliform organisms may often cause defects in cream (Pont 1935*b*) and were frequently associated with degrading at 21°C in these experiments, other types of Gram-negative rods, particularly psychrophilic types, were of greater importance at 15·5°C.

As many of the spoilage organisms tend to die out in acid cream, the occurrence of numbers below the expected threshold level may not preclude the possibility that off-flavours had been produced by such organisms before their numbers decreased. However, several creams examined in these experiments had quite high numbers of acid-producers and very few other types of organisms at any time before degrading. Also, the addition of an acid-producing organism to suppress the growth of undesirable types of bacteria was found to cause earlier degrading due to fat deterioration at low pH. For these reasons a test which would indicate the presence or previous presence of large numbers of spoilage organisms could be of advantage in determining the reason for cream degrading.

To ensure good quality cream after 3 days' storage at the farm it would seem that some cooling is essential. However, storage at 5°C frequently caused deterioration of cream due to oxidative or hydrolytic changes of the fat. McDowall (1953, p. 272) noted that oxidative changes may occur in cream stored below 4·5°C, and several American workers (e.g. Peters, Kester, and Nelson 1953; Crowe 1955) have reported increased water-insoluble acid values following low-temperature storage. Storage at 10°C allowed slow bacterial growth which was sufficient to counter oxidative changes taking place at the lower temperature. Provided the initial level of contamination in the cream was kept reasonably low, there was insufficient bacterial growth at 10° to cause deterioration of the cream within 4 days. Although most of the cream samples examined in these experiments remained of choice quality during storage at 10°C for 3 or 4 days, samples of refrigerated farm cream taken at factory receiving platforms have frequently contained excessively large numbers of psychrophilic organisms. Again, for this reason, it would seem that some test of the bacteriological quality of cream is desirable. When cream is degraded, grading comments cannot be relied on to give an indication of the reason for degrading.

Variations in the fat content of the cream samples between 35 and 45% did not appear to have any influence on bacterial growth rates or grading, but one cream with a fat content of 30% became curdy after a high bacterial count was reached.

Copper contamination is known to catalyse oxidative changes in dairy products. Estimations for copper content were made on only a few cream samples examined in these experiments, but the low values obtained in these results, the reports of the condition of the tinning of the equipment on the farms from which samples were taken, and the average values for copper content in Queensland farm cream in other experiments in this laboratory suggest that copper contamination was not sufficient to cause oxidation during the relatively short duration of storage of these cream samples. Further work on oxidative changes in relation to cream quality is proceeding.

V. ACKNOWLEDGEMENTS

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