

MICROBIOLOGICAL SURVEY OF BUTTER IN MADRAS

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## INTRODUCTION

Butter is a water-in-fat emulsion with fat as the continuous phase. Manufacture of butter constitutes a major branch of dairy industry. It is the chief means of conserving the valuable nutritive properties of milk fat. In India it is an intermediate stage in the manufacture of ghee.

The value of butter is governed by its quality, physical appearance, texture, flavour and chemical composition. Besides, the product should be free from pathogens and should possess good keeping quality.

In India, desi method of butter making is widely practised for the production of ghee. Creamery method of butter manufacture is less common in India. Total annual production of butter in India is estimated at 19.37 lakh maunds. Of this, approximately 7.4 percent (1.43) lakh maunds, is creamery butter and 92.6 percent (17.94 lakh maunds) desi butter (Marketing Report, 1957).

As the dairy industry in our country is not yet developed to manufacture butter on a factory scale, to meet the market demands desi butter has to be pooled from very many sources. Since the method of manufacture is not controlled and as it has to be pooled from small producers from distant places, by the time it reaches the city market, butter undergoes pronounced deterioration due to lack of care on transit and the time lag before it reaches the consumer. Such butter will be highly sour, have high microbial content and develop rancidity very quickly. The acidity of butter will be transmitted to ghee manufactured out of it, thereby affecting its flavour and keeping quality.

The deterioration of butter results in much wastage of valuable food in addition to the economic loss suffered by the butter producers. Many factors are responsible for the deterioration of butter. Microbial spoilage is one of the important factors in the deterioration and the reduction in keeping quality of butter.

## REVIEW OF LITERATURE

### Total count.

Microorganisms have ample opportunity to gain access to butter during its manufacture if proper hygienic care is not exercised. The growth of undesirable organisms in butter is a major cause of its deterioration during storage. Bacterial content of fresh butter is extremely variable and depends primarily on the method of manufacture. Since microorganisms play a significant role in the development of defects in butter, considerable importance is attached to their number and type.

One hundred and fifty five samples of butter were examined by De Soriano Angela (1942). The total count varied from 30 to 22,500,000 per ml. Tanner (1944) studied 25 samples of butter and reported the average number as 5,700,000 per gram. Souto et al (1946) found that 60 out of 366 samples of butter analysed to contain one million or less microorganisms, 190 between 1 and 30 million and 116 more than 30 million organisms. Krishnaswamy and Laxminarayana (1943) stated that desi butter made from boiled soured milk and stored for one to two weeks gave average counts of about 200,000 bacteria per ml. Creamery butter made from pasteurized cream and 24 to 48 hours old gave counts about 2 million bacteria per ml. Ozawa and Kondo (1952) reported the maximum bacterial count in Japanese butter as 13,000,000 per gram. Grosso and Bergamini (1956) in their bacteriological investigation of 201 samples of market butter had the following counts. Bacterial counts over 5 million per cc were found in 71.5 percent of the samples examined and 23.3 percent had counts of 30 millions per cc. Vecchio V. Del and D'Arca (1957) found that nearly 40 percent of the 110 samples

subjected to microbiological examination contained above 50 million bacteria per ml. Sapio (1958) examined 104 butter samples from the Naples market. Of these 40 percent had total bacterial counts of 1 to 5 million per ml, while 32 percent of the non-commercial samples had counts of 5 to 40 million per ml. Lilov (1960) studied 40 samples of ripened cream butter and found that in most cases the total counts exceeded 1,000,000 per gram. Roughley and McLeod (1960) in their microbiological survey stated that 59.4 percent out of 1,058 samples examined had a total count of 100 to 50,000 per gram; 14.1 percent revealed between 50,000 to 100,000 per gram; 12.6 percent were in the range of 100,000 to 300,000 per gram; and 14 percent had more than 300,000 per gram. Krishnamurti (1961) made a study on Indian market butter. According to him the average count in desi butter was 12,675,000 per ml, the average plate count of unsalted ripened cream butter was 3,527,000 per ml and the average plate count of sweet cream salted butter was 26,000 per ml. Rasic et al (1962) reported that the total counts of butter sampled immediately after manufacture ranged generally from 200,000 to 600,000 per gram.

In controlled experiments, Parfitt (1934) inoculated pure cultures of yeast or bacteria into sterile cream and then churned into sterile jars. The number of organisms per ml in the unsalted butter always was smaller than the numbers in the cream. The wash water removed considerable number of organisms. The bacteria in butter ranged from 0.3 to 10.5 with an average of 3.9 percent of the count in cream. Pont (1941) observed that there was no significant correlation between total bacterial count, salt or moisture. Jamieson and Chen (1943) reported that the counts of bacteria were greatest in the containers that had been most frequently opened.

Elliker (1944) reported that during churning of cream ripened by culture, the percentages of the total bacteria in different fractions were, butter 0.75, butter milk 91.57 and wash water 7.68. Hoecker and Hammer (1945) reported that the lowest counts were obtained in butter having salt well distributed. Paskert (1957) after systematic examination of butter noted a lack of correlation between bacteriological tests and the keeping quality of butter as judged by organoleptic tests.

Hood and White (1931) suggested that pasteurized cream butter should contain less than 25,000 bacteria per ml. Moir and Russel (1937) reported that the export butter should have a total count of less than 50,000. Adam (1956) proposed that the bacterial counts other than lactic acid bacteria should be less than 25,000 per gram. Wilster (1957) suggested for high efficiency in butter manufacture the bacterial count should be less than 5,000 and none of the yeast and moulds.

#### Yeast and mould count.

Yeast and mould counts are made to obtain information on the sanitary efficiency of plant operation. In addition it helps to find out the type of deterioration.

De Soriano Angela (1942) reported the mould count to range from 0 to 100,000. Claydon (1943) reported that the mould content of butter made from pasteurized cream was lower than that made from raw cream. According to Krishnaswamy and Laxminarayana (1948) desi butter made from boiled soured milk and stored for one to two weeks gave counts of 5,000 yeasts and moulds per ml, whereas creamery butter made from pasteurized cream and 24 to 48 hours old gave average counts of 300 to 600 yeasts and moulds per ml.

Vecchio V. Del and D'Arca (1957) reported the analysis of 110 samples. Of which, 15 percent had more than 1 million mould spores per ml. The average yeast counts reported by Rogick and Dias (1959) for freshly made butter were 3,100 per ml and 315 per ml for inadequately and adequately pasteurized samples respectively. Roughley and McLeod (1960) analysed 1,058 samples and reported that 64.5 percent of samples had 20 or less; while in 35.57 percent the count was above 20 per gram. Krishnamurti (1961) recorded average yeast and mould counts in desi butter as 1,485,000 per ml. The average count in the ripened cream butter samples was 466,000 per ml. The average yeast and mould count of sweet cream salted butter was 3 per ml.

Combs and Eckles (1947) observed that Geotrichum candidum and Penicillium caused rancidity in butter. Voitkevich (1928) stated that high humidity favoured the development of moulds. Increase in number of mould was correlated with high acidity. Macy and Richie (1929) found that high yeast counts generally accompanied high mould counts but no consistent relationship could be traced between mould or yeast counts and the quality of fresh butter; whilst taken as a group the samples of butter with the lower mould, yeast and total counts showed a tendency towards slightly better quality than those with high counts. According to Nyiredy et al (1934) excessive yeast and mould count suggested churn contamination. Vernon (1935) reported colour defects in butter caused by fungi. Wood and Thornton (1935) and Moir and Russel (1939) stated that yeast and mould count was not the only criterion for measuring creamery sanitation. George (1941) established a relationship between the viable mould count of cream and the mycelia count of corresponding butter. Davies (1941) observed that Oidium and Oospora caused butter to become oily and

develop a fishy odour while Penicillium, Monilia and Cladosporium caused ketonic rancidity. Block (1941) stated that high moisture content, low salt and high storage temperature favoured yeast and mould growth. Claydon (1942) considers that mycelia count is a reasonably accurate measure of the quality of cream for butter making and of commercial butter. Swartling et al (1962) observed that mould infection occurred via air, equipment and staff.

Hood and White (1931) suggested that pasteurized cream butter should contain less than 500 yeasts and moulds per ml. Muller and Nichols (1950) proposed the standard for yeast and mould count not to exceed 100 per ml. According to Hammer (1957) the objective should be to have all samples from a plant to show less than 1 per ml. According to Druce and Thomas (1959) yeast and mould counts were one among the most useful and economical tests for providing information on the standard of hygiene during manufacture and also gave some indication of the potential keeping quality of the butter. They recommended that the yeast and mould counts should be less than 50 per gram.

#### Lipolytic count.

One very potent disturbing factor in the keeping quality of butter is the secretion of lipases by microorganisms and the consequent lipolysis being governed by different conditions from those pertaining to the natural lipase of milk.

Stark and Scheib (1936) investigated the correlation between the spoilage of butter and the presence of fat splitting organisms. They found a good correlation between the keeping quality and the numbers of lipolytic and caseolytic organisms in butter. Of 468 strains which split milk fat or tributyrin and isolated from butter, 96 percent attacked tributyrin and 77 percent fat. Moir and

Russel (1937) laid the bacteriological standard for lipolytic organisms to be less than 1,000. De Soriano Angela (1942) reported that the lipolytic count in 155 samples ranged from 0 to 2,500,000 per ml. Angela Soriano (1948) studied 103 butter samples bought in Buenos Aires for lipolytic bacteria of which 52.4 percent were contaminated with lipolytic bacteria.

The rate of lipolysis of butter by Candida lipolytica (Harrison), Pseudomonas fluorescens (Migula) and an unidentified yellow mould isolated from rancid butter was studied by Downes (1959). Of the three, C.lipolytica appeared to be the most active lipolytic agent producing considerable amounts of free fatty acids at a rapid rate. Petula and Kangaskarki (1959) reported that high lipolytic activity in butter with a high amount of free fatty acids carried a rancid flavour.

#### Coliform organisms in butter.

The coliform counts of butter indicates the sanitary efficiency of the factory, for they are destroyed at temperatures such below 200°F. Their presence in butter means that at some stage in the manufacturing process following pasteurization, contamination has taken place.

Amaral Rogick (1942) on his examination of butter for the presence of coliform organisms showed that 30 out of 43 samples of unsalted butter were positive and all 9 samples of salted butter were negative. The maximum count was 160,000 per ml. Tanner (1944) found that six of the 24 samples examined were positive for Escherichia coli. Krishnaswamy and Laxminarayana (1946) observed that 50 percent organisms in creamery butter was coliform. Krishnaswamy (1949) stated that the occurrence of coliform organisms was next in order to lactobacilli and streptococci. Thomson (1950)

reported that of 719 samples of export salted butter tested for coliform contamination, 19.1 percent gave confirmed results using MacConkey broth as presumptive medium. Ozawa and Kondo (1952) observed that 21.8 percent of samples examined had more than 10,000 coliform organisms per cc. Inoue Norimasa (1954) studied the frequencies of appearance of the coliform bacteria and enterococci in butter. Out of 48 samples Escherichia coli type was found among 24 samples. Aerobacter group, chiefly Aerobacter aerogenes type was found in butter dilution as high as  $10^{-4}$  to  $10^{-5}$  but was detected generally in dilutions of  $10^{-2}$  to  $10^{-3}$ . Grosso and Bergamini (1956) reported that 21.8 percent of samples contained more than 10,000 coliform organisms per cc. Vecchio V. Del and D'Arca (1957) found that 17 percent of the 110 samples subjected to microbiological examination contained 1 to 10 million coliforms per ml. Sapio (1958) stated that the coliform count was less than 100 organisms per ml in 91 percent of non-commercial samples. 30 percent of the commercial samples had the count between  $10^{-4}$  and  $10^{-5}$  per ml. Rogick and Dias (1959) reported that the average number of coliforms per ml was 41,820 for fresh inadequately pasteurized unsalted butter, whereas that of fresh pasteurized unsalted butter was 102. Lilov (1960) found that out of forty samples examined, 6.6 percent was positive for coli-aerogenes. Roughley and McLeod (1960) observed that in 76.9 percent of the samples the coliform count was 10 per gram or less and in 23.1 percent the coliform count was above 10 per gram. Krishnamurti (1961) reported that in 36.84 percent of desi butter samples tested, coliform type was detected. On the contrary, only 7.02 percent of ripened cream butter samples was positive for coliform that too in dilutions not exceeding 100. None of the sweet cream salted butter samples was positive.

Hammer and Yale (1932) reported that *Aerobacter* species were more harmful to butter than *Escherichia*. However they are easily killed at moderate temperature and so provide a bacterial index of sanitation. According to Nyiredy et al (1934) high coli count indicated insufficient pasteurization of cream. Long et al (1944) observed that heat resistant coliform organisms were frequently obtained from butter. Of 116 cultures isolated from butter, 74 survived 20 minutes at 61.7°C, 48 survived 30 minutes and 28 survived 40 minutes. None of the *aerobacter* cultures obtained from the same dairy products was heat resistant. The results indicated that if *Escherichia* cultures were obtained from pasteurized dairy products, their heat resistance should be determined before assuming that pasteurization was inadequate or that subsequent contamination had occurred. Madsen (1959) stated that the presence of coliform organisms could be correlated with inadequate pasteurization or insanitary factory conditions.

Moir and Russel (1937) reported that butter to be of satisfactory quality should be free from coliform organisms. The Queensland Butter Improvement Service adopted the bacteriological standard for coliform organisms as a negative test in 1/10th ml (Muller and Nichols, 1950). Druce and Thomas (1959) recommended to have a standard of less than 10 coliform organisms per gram.

According to Rajnath Singh (1949) high coliform counts were generally associated with poor keeping quality of butter. However, he pointed out that low counts did not necessarily mean butter of good keeping quality. Newman (1950) considered that *Escherichia coli* could not be regarded as a conclusive index to sanitation.

Acidity in butter.

The quality of butter is significantly affected by titrable acidity. A very high titrable acidity in butter indicates uncontrolled ripening of cream and results in chemical deterioration on storage. Desi butter has a high titrable acidity because of uncontrolled ripening of milk. Even though the acid reaction may not be favourable for bacterial growth, desi butter rapidly undergoes pronounced rancidity and cheesiness either because the bacteria are aciduric or because of the lipolytic action of yeasts and moulds.

White et al (1929) stated that butter made from cream with acidity of 0.31 percent or lower kept well than butter from cream of higher acidity. Dienert (1932) in an analysis of 18 samples of butter from different parts of France, observed that acidity and E.coli content were roughly inversely proportional. Haglund (1933) stated that highly ripened butter deteriorated more rapidly than butter ripened to a lower degree. According to Wiley (1939) acidity, starter organisms, salt and low pasteurization temperature favoured fat oxidation. White (1944) showed that the principal flavour defects noted in high acid butter were metallic, sour or acid flavour and fishiness. Grant et al (1948) reported that the best tests for the keeping quality were free acidity, total acidity and fluorescence of the serum. Young S.Ven (1951) found that sour butter was of poor keeping quality. Crittal (1957) studied the butter stored for six months at 10°F and found that those in the pH range 7.8 to 8.2 had the best keeping quality and flavour. No correlation between pH and bacterial development after storage was observed. Milohnofa et al (1964) reported that neither the acidity nor the serum pH were suitable criteria for assessing the deterioration of butter quality.

Knudsen et al (1939) observed that the number of bacteria and pH followed each other very closely, whereas the titrable acidity continued to rise after the number of bacteria and pH reached a constant value. Rangappa and Achaya (1948) reported the optimum level of curd acidity to be between 0.9 and 1.1 percent for the highest yield and quality of butter. Krishnamurti (1961) reported that titrable acidity is a more reliable measure to assess the quality of butter than pH. Raghavan and Kachroo (1962) reported that the percentage of acidity of fresh butter was higher in the buffalo milk butter than in that of cow milk butter.

According to Hunziker (1940) for satisfactory keeping quality without chemical deterioration, titrable acidity of salted butter should not exceed 0.05 to 0.06 percent lactic acid. In case of unsalted butter acidity from 0.12 to 0.15 percent can be permitted because it will improve the keeping quality by retarding bacterial activity.

#### Acidity in ghee.

There is a direct relationship between acidity of ghee and acidity of butter. According to Patel et al (1949) acidity of ghee is controlled by acidity of butter and ghee manufactured by desi method had a poor keeping quality. Paul and Anantakrishnan (1949) observed that ghee produced from milk not heated and curd stored for 3 to 4 days had high acidity. This was due to active mould growth on the cream layer. Rangappa and Achaya (1948) reported high acidity in ghee from unprocessed and inefficiently processed milk. They observed that ghee prepared by desi process and examined fresh had an acidity of about 0.2 percent expressed as lactic acid. The transfer of butter acidity to ghee was more than one half in the case of unprocessed milk

butter whereas in boiled-milk butter this was less than one half in 3 days storage of butter. Both increased to an appreciable level with the number of days of storage.

Lalitha and Dastur (1953) stated that high acid ghee developed oxidative spoilage rapidly. Ghee with an initial acidity upto 2.5 oleic acid developed further acidity only slowly. Raghavan and Kachroo (1962) stated that high acidity in market ghee made from collected butter was generally accompanied by a rancid smell.

## SCOPE OF INVESTIGATION

It may be seen from the review of literature that careless methods of manufacture and handling of butter is responsible for the large numbers and types of organisms in it. The present investigation was taken up to study the following:

1. The microbiological quality of the different types of butter.
2. The different types of deterioration taking place in butter.
3. The quality of butter and its impact on the quality of ghee prepared from it.
4. To suggest improvements on the preparation of butter for ghee making.

## MATERIALS AND METHODS

### (i) Sampling.

Desi butter: In this study, desi butter available in the local retail stores was used. The points of butter production are more than 200 miles away from the city and it is claimed that the butter which comes daily in tins reaches the city market within two days from the time of collection. But the samples used in these experiments were secured after the tins were opened and exposed for sale. Before sampling, butter was thoroughly mixed and 100 gms of the sample was packed and taken to the laboratory and kept in a refrigerator at 5°C till it was analysed.

At the time of conducting the experiment the sample was removed from the refrigerator and opened under aseptic conditions. With a sterile spatula the surface of butter was scraped off and with another sterile spatula, small dabs of butter from six different points of the mass were taken and transferred to a sterile ground glass stoppered sampling bottle of 8 oz. capacity.

Creamery butter: Ripened cream butter and sweet cream salted butter of creamery were collected from the retail stores. Ripened cream butter samples were wrapped in butter paper. Sweet cream salted butter samples were sold in 100 gms of prints neatly wrapped in parchment. The same sterile precautions adopted for desi samples were carried out.

### (ii) Bacteriological analysis.

After a preliminary examination for the physical condition, estimations were made for total bacterial count, yeast and mould count, lipolytic count and presumptive coliform test. Total bacterial count was estimated by standard plate count. Hammer (1957) has

suggested that any agar satisfactory for plate counts in milk can be used for butter. So tryptone glucose extract agar was used as medium.

Preparation of samples and procedure for plating were followed as per Standard methods for the examination of dairy products (1960).

Counting of plates: After incubation for 48 hours at 35°C colonies were counted with the aid of Quebec Colony counter. Consistently spreader free plates with colonies between 30-300 were selected. All the colonies including the pinpoint colonies were counted. The average of duplicate plates were expressed as standard plate count per ml.

Yeast and mould count: For estimating yeast and moulds Bacto malt agar was used. Sterile distilled water warmed to 45°C was used for dilution and the procedure followed as outlined in Standard methods for the examination of dairy products (1960).

Lipolytic count was estimated using tryptone glucose extract agar (1000 ml) to which was added 20-40 ml of melted butter fat and 5-10 ml 0.1 percent aqueous solution of Nile-blue sulphate. Lipolytic bacteria hydrolyse the fat globules producing a blue colour around and beneath the colonies. The unhydrolysed fat globules appear pink, due to the action of Nile-blue pink, an impurity present in Nile-blue sulphate.

Presumptive coliform test: The method of preparing the plates was the same as in standard plate method described previously, except that after the solidification another 3-4 ml of the same medium was distributed to cover completely the surface of the already solidified medium to inhibit surface colony formation. Eosin-methylene blue agar (EMB-agar) was used to estimate the coliform organisms.

The plates were incubated at 37°C for 24 hours after which the developed red colonies not less than 0.5 mm diameter were counted.

(iii) Samples collected for bacteriological examination were subjected to the estimation of titrable acidity in butter and acidity in ghee.

Determination of titrable acidity: In a conical flask 18 gms of the sample to be examined was taken. Previously boiled hot distilled water of 90 ml was added to it and 1 ml of 1 percent phenolphthalein indicator. Titration was done while hot against N/50 sodium hydroxide solution from a burette. Each ml of the alkali solution used is equivalent to 0.01 percent lactic acid. The procedure followed in the study is recommended by Nissen (1931) and Ling (1956),

Acidity in ghee: Small quantity of butter was converted into ghee and about 20 gms was weighed in a porcelain dish and to it 50 ml of neutral 95 percent alcohol was added. 1 ml of 0.5 percent phenolphthalein was added. The contents were heated to boiling and thoroughly mixed and titrated against 0.1 N sodium hydroxide. The result was expressed as percent of oleic acid.

## RESULTS AND DISCUSSION

Since microorganisms play a significant role in the development of defects in butter, a study was made to estimate the total count.

Table I.

Bacteriological quality of desi butter.

Bacterial range in million.	Percentage of samples.	Average plate count for the range (per ml)
0 to 1	27.27	610,000
1 to 3	31.82	1,700,000
3 to 5	9.09	3,600,000
Over 5	31.82	8,200,000

Table I represents the bacteriological quality of desi butter. Out of a total of 22 samples analysed only 27.27 percent had a count of less than one million. It is seen that 72.73 percent of samples had a count of more than one million and 31.82 percent of samples had a count of more than 5 millions. The average plate count was 3,600,000 with a minimum of 240,000 and a maximum of 10,000,000.

Table II.

Bacteriological quality of unsalted ripened cream butter.

Bacterial range in million.	Percentage of samples.	Average plate count for the range (per ml)
0 to 1	57	250,000
1 to 3	43	1,600,000

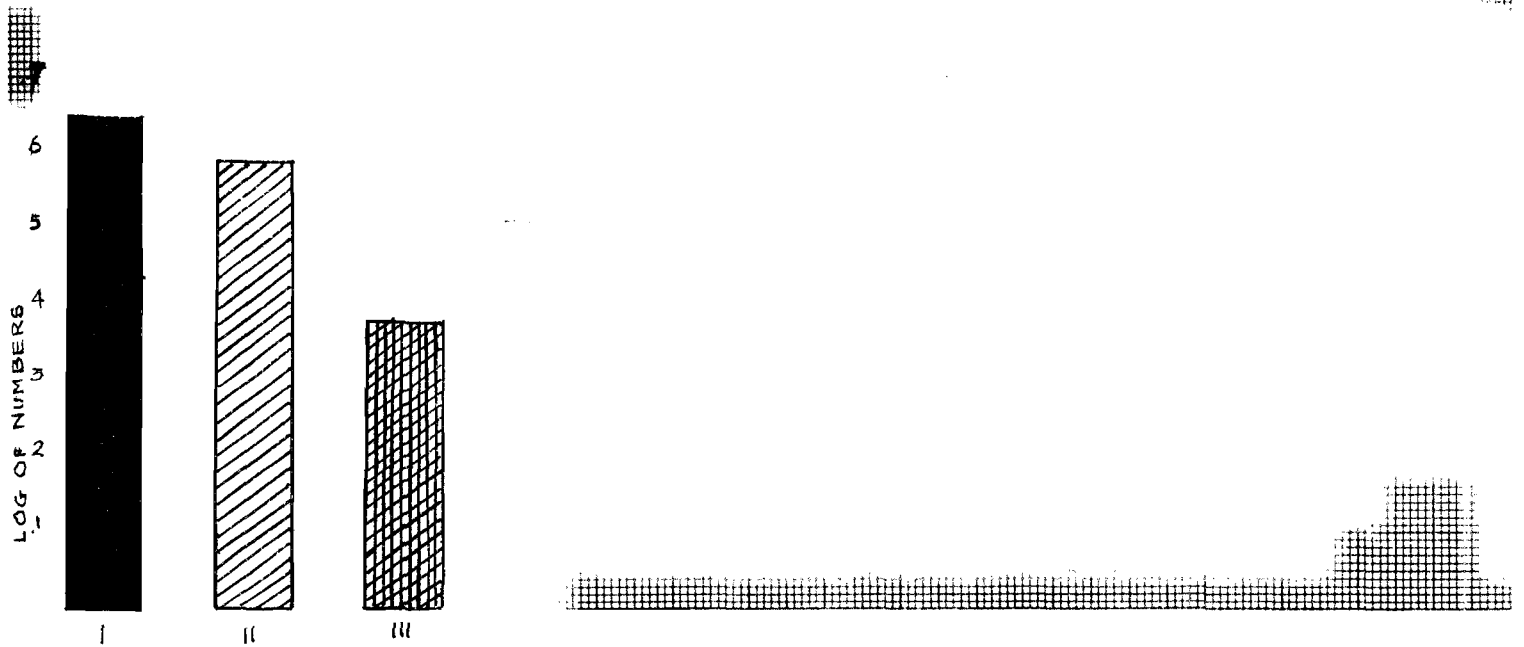
Table II presents the bacteriological quality of ripened cream butter. It will be seen from the table that all the samples have a count of less than 3 millions and 57 percent of the samples analysed have a count of less than one million thereby indicating that the

GRAPH I STANDARD PLATE COUNT COUNT IN LOGARITHMS

I DESI BUTTER

II RIPENED CREAM BUTTER.

III SWEET CREAM SALTED BUTTER.



bacteriological quality of ripened cream butter is better than desi butter. The average plate count of 7 samples of ripened cream butter, was 960,000 with a minimum of 150,000 and a maximum of 2,200,000.

Table III.

Bacteriological quality of sweet cream salted butter.

Bacterial range in thousand.	Percentage of samples.	Average plate count for the range (per ml)
0 to 5	80	1,700
5 to 10	10	7,500
Over 10	10	48,000

The average plate count of 10 samples of sweet cream salted butter was 6,900 with a minimum of 330 and a maximum of 48,000. In contrast to desi and ripened cream butter, sweet cream salted butter has strikingly a very low count. The bacteriological quality is uniform and the count is only in thousands as compared to that of other two. The present findings are in agreement with the report of Krishnamurti (1961) who observed that bacterial count in desi butter is maximum and moderate in ripened cream butter and least in sweet cream salted butter.

The three kinds of butter studied, viz. desi, ripened cream and sweet cream salted butters differ in their bacterial content. Table IV and Graph I indicates the difference in their bacteriological quality.

Table IV.

Bacteriological quality of different types of butter.

Kinds of butter.	Average plate count (per ml).
<u>Desi</u> butter	3,600,000
Ripened cream butter	960,000
Sweet cream salted butter	6,900

From the table it is seen that from a sanitary standpoint sweet cream salted butter is the best and desi butter the poorest. The ripened cream unsalted butter is mediocre in quality.

In U.S.A. yeast and mould count is the standard test for checking factory sanitation. So a study has been made to investigate the occurrence of yeasts and moulds in different kinds of butter. All the 20 samples of desi butter analysed reveal yeasts and moulds. The average count is 510,000 with a minimum of 35,000 and a maximum of 1,100,000. Table V shows the frequency distribution of yeast and mould count in desi butter.

Table V.

Yeast and mould count in desi butter.

Range.	Percentage of samples	Average yeast and mould count (per ml)
0 to 100,000	25	63,000
100,000 to 1,000,000	55	410,000
Over 1,000,000	20	1,300,000

Out of a total number of 20 samples analysed for yeast and mould count 75 percent of the samples had a count of more than 100,000 and 20 percent had more than one million.

Table VI.

Yeast and mould count in ripened cream butter.

Range.	Percentage of samples.	Average yeast and mould count for the range (per ml)
0 to 100,000	57.2	57,000
100,000 to 1,000,000	42.8	490,000

All the 7 ripened cream butter samples studied reveal yeasts and moulds. The average count is 240,000 with a minimum of 18,000 and a maximum of 580,000. Table VI represents the frequency distribution of yeast and mould count in ripened cream butter. All samples of ripened cream butter had a count of less than one million and 57.2 percent of samples had a count of less than 100,000, thereby indicating that ripened cream butter is better than desi butter so far as yeast and mould count is concerned. The average yeast and mould count of 10 samples of sweet cream salted butter is 21 with a maximum of 100. No yeasts and moulds were present in 3 samples and 6 samples had a count of less than 10.

Table VII.

Yeast and mould count in butter and its relationship to plate count.

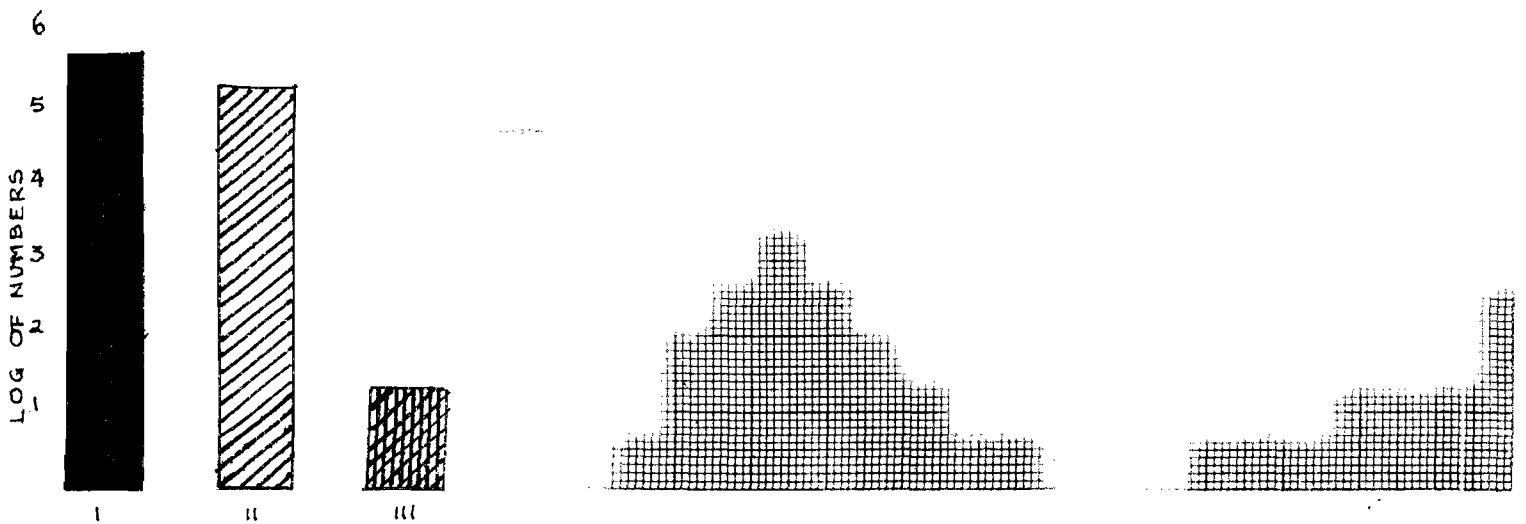
Kinds of butter.	Average yeast and mould count (per ml)	Average plate count (per ml)
<u>Desi</u> butter.	510,000	3,600,000
Ripened cream butter.	240,000	960,000
Sweet cream salted butter.	21	6,900

GRAPH II YEAST AND MOULD COUNT COUNT IN LOGARITHMS

I DESI BUTTER

II RIPENED CREAM BUTTER

III SWEET CREAM SALTED BUTTER



The yeast and mould count in different kinds of butter is consolidated in Table VII and in Graph II. It is seen that the yeast and mould count is maximum in desi butter; so also the standard plate count. The present findings are in agreement with the report of Krishnaswamy and Laxminarayana (1948) who observed that desi butter contains more of yeasts and moulds because of its acid environment. In ripened cream butter the yeast and mould count is lower; so also the standard plate count. They are the least in sweet cream salted butter.

Table VIII.

Lipolytic count in desi butter.

Range.	Percentage of samples.	Average lipolytic count for the range (per ml)
0 to 10,000	31.83	5,100
10,000 to 100,000	54.54	24,000
Over 100,000	13.63	310,000

As the lipolytic organisms determine the potential keeping quality of butter a study has been made on their incidence. The average lipolytic count of 22 samples of desi butter is 57,000 with a minimum of 350 and a maximum of 690,000. Table VIII shows the lipolytic count in desi butter.

GRAPH III LIPOLYTIC COUNT COUNT IN LOGARITHMS

I DESI BUTTER

II RIPENED CREAM BUTTER

III SWEET CREAM SALTED BUTTER.

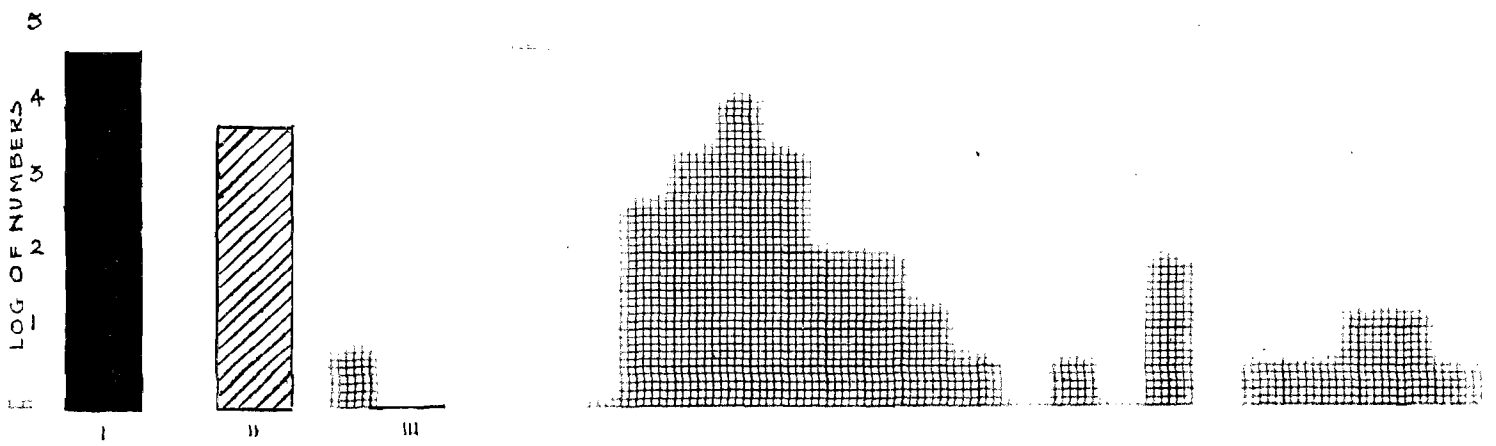


Table IX.

Lipolytic count in ripened cream butter.

Range.	Percentage of samples.	Average lipolytic count for the range (per ml)
0 to 10,000	85.71	3,000
10,000 to 100,000	14.29	15,000

The average lipolytic count of 7 samples of ripened cream butter is 4,700 with a minimum of 650 and a maximum of 15,000. The frequency distribution of lipolytic count in ripened cream butter is shown in Table IX. From the table it is seen that 85.71 percent of the samples analysed had a count of less than 10,000 as compared to only 31.83 percent of desi butter samples, thereby indicating that ripened cream butter is better than desi butter in lipolytic count. Ten samples of sweet cream salted butter had a count of less than 1. No lipolytic colonies were present in 8 samples. Only 2 samples revealed lipolytic count of 4 and 1 respectively.

Table X.

Lipolytic count in butter and its relationship in plate count.

Kind of butter.	Average lipolytic count (per ml)	Average plate count (per ml)
<u>Desi</u> butter	57,000	3,600,000
Ripened cream butter.	4,700	960,000
Sweet cream salted butter.	Less than 1	6,900

The results of lipolytic count in different kinds of butter is consolidated in Table X and in Graph III. The average plate count

of 22 samples of desi butter subjected to lipolytic count is 3,600,000; in ripened cream butter it is 960,000 and in sweet cream salted butter 6,900. There tends to be a positive correlation between standard plate count and lipolytic count.

In Australia total plate count and coliform tests are considered necessary in addition to the yeast and mould count as the coliforms are useful indicator organisms of good factory hygiene. So a study has been made on the incidence of coliform organisms in various butters.

Table XI.

Coliform count in desi butter.

Range.	Percentage of samples.	Average coliform count for the range (per ml)
0 to 1,000	4.55	300
1,000 to 10,000	54.54	2,600
10,000 to 100,000	31.82	53,000
Over 100,000	9.09	1,600,000

The average coliform colonies per ml in 22 samples of desi butter is 160,000 with a minimum of 300 and a maximum of 2,700,000. The frequency distribution of coliform organisms in desi butter is shown in Table XI. From the table it will be seen that 95.4 percent of samples have a count of more than 1,000.

GRAPH IV COLIFORM COLONIES COUNT IN LOGARITHMS

I DESI BUTTER

II RIPENED CREAM BUTTER

III SWEET CREAM SALTED BUTTER

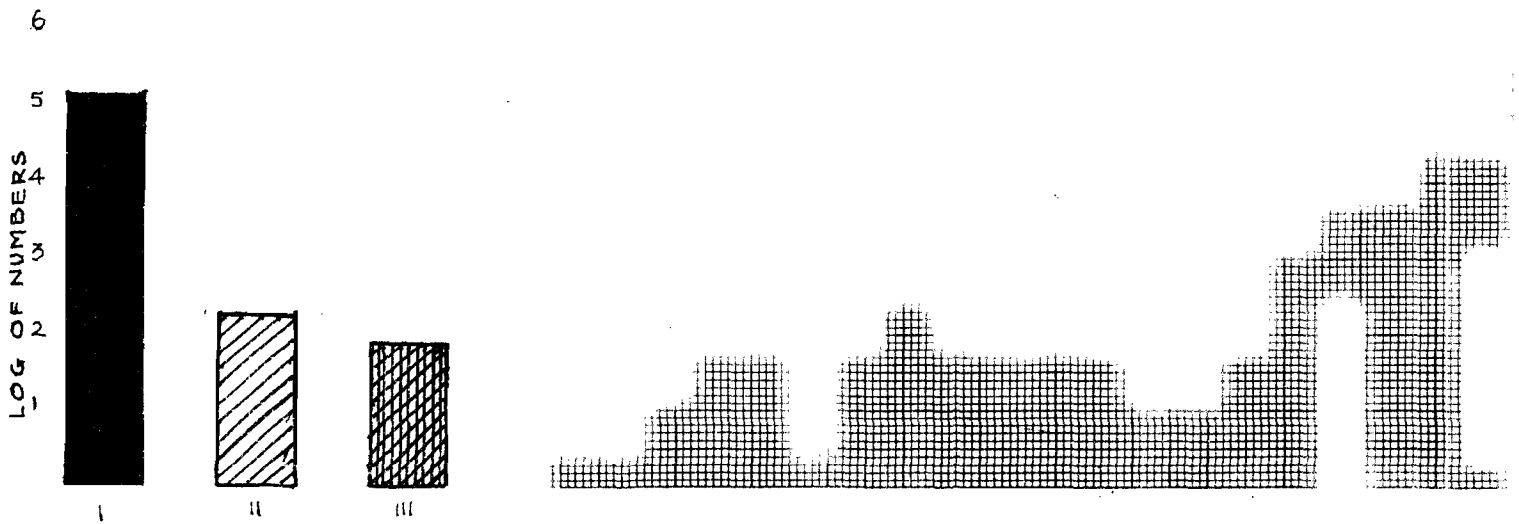


Table XII.

Coliform count in ripened cream butter.

Range.	Percentage of samples.	Average coliform count for the range (per ml)
0 to 1,000	85.71	210
1,000 to 10,000	14.29	1,500

From Table XII it is seen that 85.71 percent of a total of 7 samples of ripened cream butter analysed have a count less than 1,000 thereby indicating that ripened cream butter is better than desi butter in bacteriological quality. The average coliform colonies per ml of 10 samples of sweet cream salted butter is 90 with a maximum of 485. Six samples revealed no coliform organisms.

Table XIII.

Incidence of coliform organisms and plate counts in different kinds of butter.

Kinds of butter.	Average coliform count (per ml)	Average plate count (per ml)
<u>Desi</u> butter.	160,000	3,600,000
Ripened cream butter.	400	960,000
Sweet cream salted butter.	90	6,900

The results of coliform organisms in different kinds of butter is consolidated in Table XIII and Graph IV. From the table it is seen that as in the case of lipolytic count there tends to be a positive correlation between standard plate count and coliform organisms.

The correlation between the onset of rancidity, total count, differential counts and acidity.

Kind of butter.	Period for developing rancidity (in days)	Average serum acidity (percent lactic)	Average free fatty acids (percent oleic)	Average plate count (per ml)	Average yeast and mould count (per ml)	Average lipolytic count (per ml)	Average coliform count (per ml)
<u>Desi</u> butter	3	0.54	2.16	3,100,000	600,000	16,000	17,000
Ripened cream butter	9	0.17	0.95	880,000	320,000	2,900	220
Sweet cream salted butter.	Over 15	0.03	0.26	7,200	23	Less than 1	44

Period for the development of rancidity in different kinds of butter was studied by keeping them at room temperature so as to find out their keeping quality in their natural marketing conditions. The three kinds of butter were kept in sterile sampling bottles with dust proof stoppers and tested organoleptically everyday. Results are furnished in Table XIV.

Sixteen samples of desi butter, five samples of ripened cream butter and nine samples of sweet cream salted butter were subjected to this analysis. Desi butter developed rancidity in 3 days, ripened cream butter in 9 days on an average and sweet cream salted butter did not develop lipolysis even after 15 days. Desi butter had a high total count, lipolytic, yeast and mould counts and coliform organisms. Ripened cream butter contained less and sweet cream salted butter the least. As the spoilage organisms had been more in desi butter, it developed rancidity quickly.

Desi butter has high serum acidity. The acidity in butter carried over to ghee is very well indicated in the table XIV. The acidity of ghee (free fatty acids) made from desi butter is highest, lowest in ghee made from sweet cream salted butter and moderate in ghee made from ripened cream butter. High serum acidity in desi butter is due to uncontrolled ripening of dahi. This high serum acidity probably makes the desi butter a selective medium for the proliferation of yeasts and moulds. It is also stated that the early development of rancidity in desi butter is due to lipolysis by moulds (Rangappa and Banerjee, 1946).

To find out the possible reason for the early deterioration of desi butter, the desi and ripened cream butters were examined for



Plate No.1.

Desi butter. Standard plate count.  
Before ageing. Dilution: 1 in 100,000.

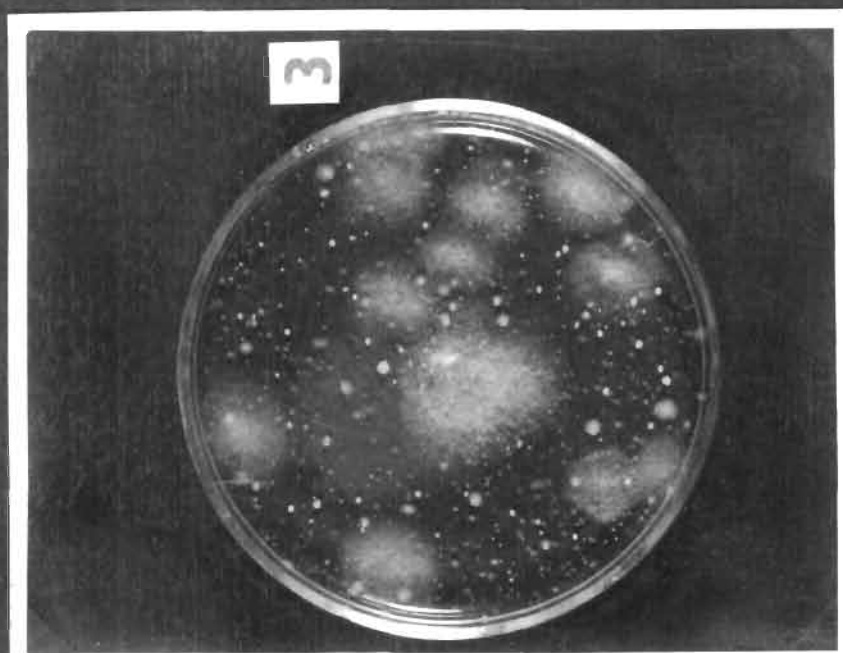


Plate No.2.

Desi butter. Standard plate count.  
After ageing. Dilution: 1 in 100,000.

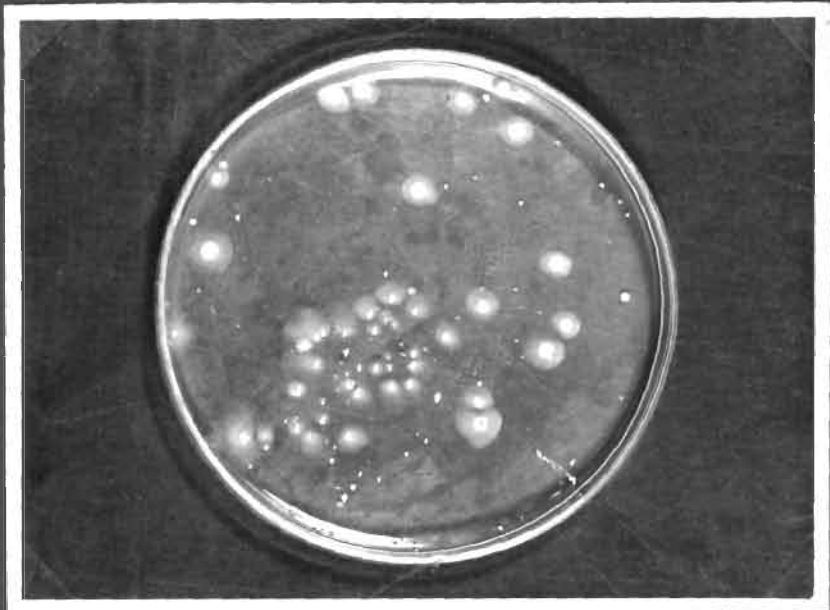


Plate No.3.  
Desi butter. Yeast and mould count.  
Before ageing. Dilution: 1 in 10,000.

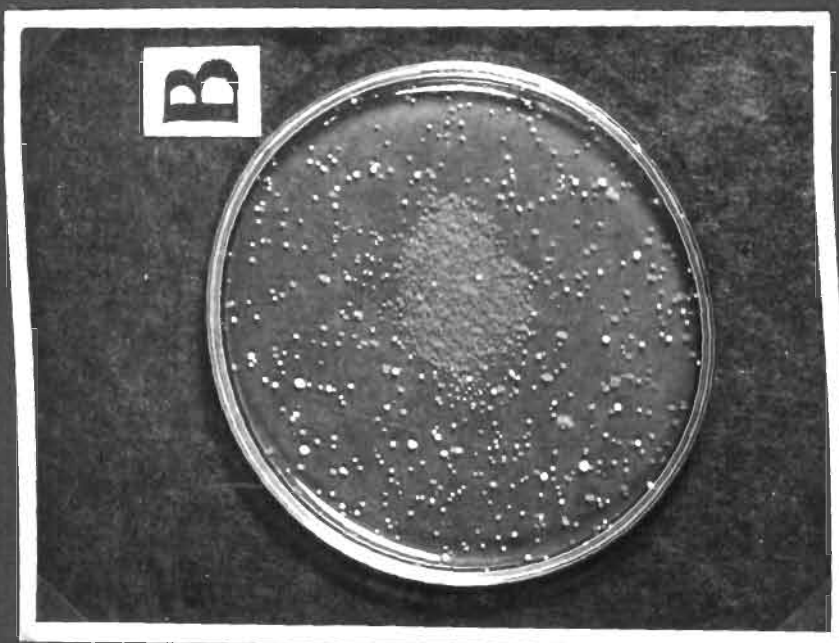


Plate No.4.  
Desi butter. Yeast and mould count.  
After ageing. Dilution: 1 in 10,000.

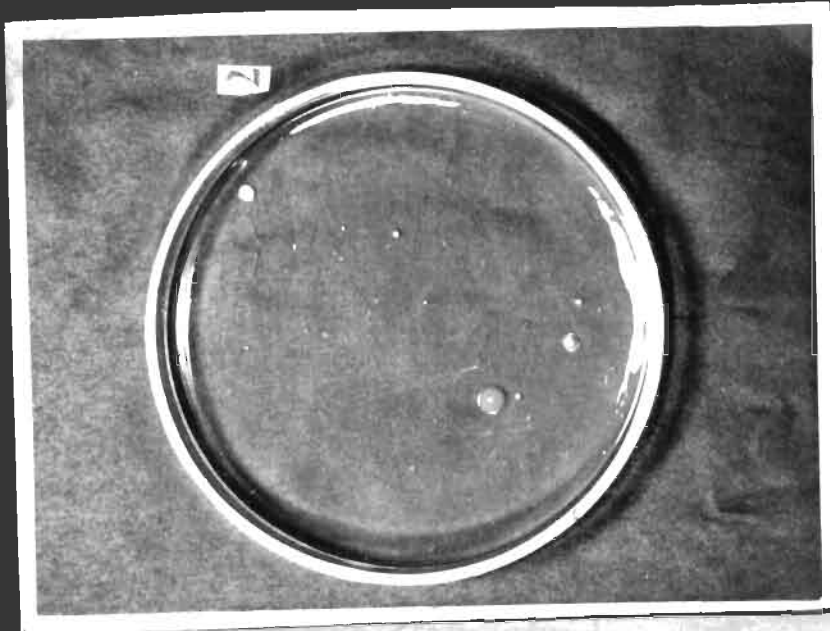


Plate No.5.  
Ripened cream butter. Standard plate count.  
Before ageing. Dilution: 1 in 10,000.

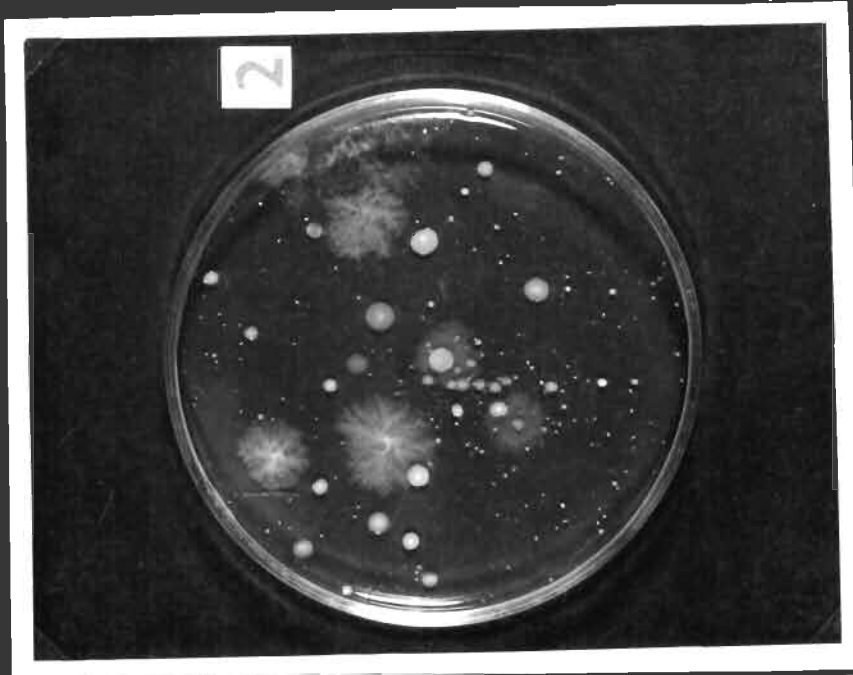


Plate No.6.  
Ripened cream butter. Standard plate count.  
After ageing. Dilution: 1 in 10,000.

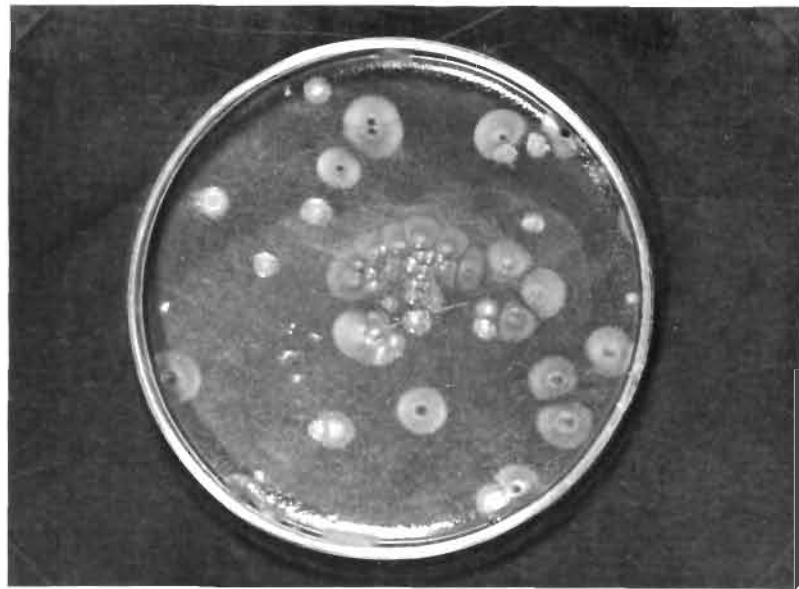


Plate No.7.

Ripened cream butter. Yeast and mould count.  
Before ageing. Dilution: 1 in 10,000.

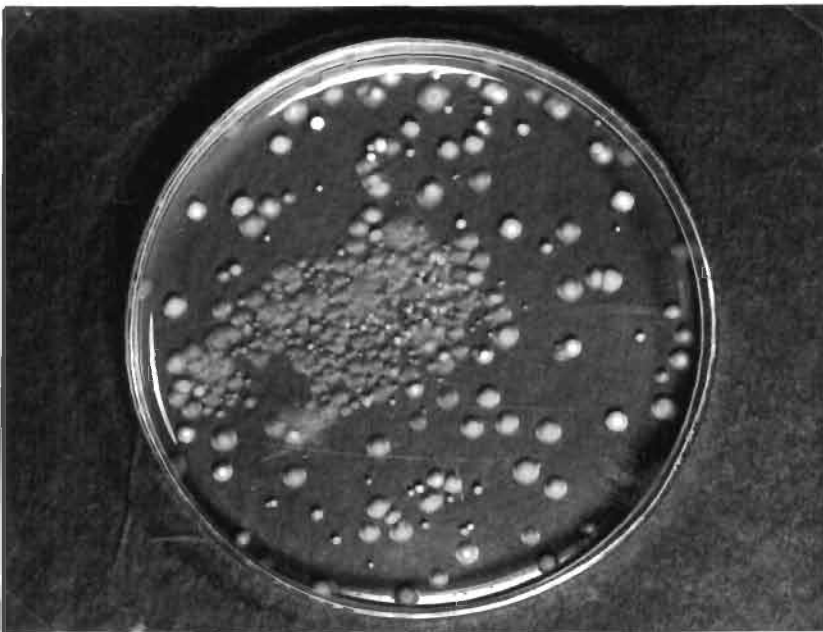


Plate No.8.

Ripened cream butter. Yeast and mould count.  
After ageing. Dilution: 1 in 10,000.

plate count, lipolytic count and yeast and mould count after ageing for one week. In sweet cream salted butter, microbial estimates were made after ageing for a fortnight. The initial bacteriological quality of the three kinds of butter was also determined before ageing. Six samples of desi butter, five samples of ripened cream butter and three samples of sweet cream salted butter were subjected to microbial estimates. Plates 1 to 8 indicate the initial microflora of desi and ripened cream butters and the microflora after ageing for 7 days in identical dilutions.

Table XV presents the results of microbial counts of different types of butter before and after ageing.

Table XV.

Microbial counts before and after ageing.

Kind of butter.	aged Number of days	Average standard plate count (per ml)		Average lipolytic count (per ml)		Average yeast and mould count (per ml)	
		Initial	After ageing	Initial	After ageing	Initial	After ageing
<u>Desi butter.</u>	7	4,300,000	48,000,000	21,000	940,000	830,000	27,000,000
Ripened cream butter.	7	680,000	21,000,000	2,900	390,000	320,000	23,000,000
Sweet cream salted butter.	15	19,000	1,600,000	nil	nil	40	5

It is seen from the table that the plate count, lipolytic count and yeast and mould count have enormously increased in desi and ripened cream butters after ageing for 7 days. In these two the total count and yeast and mould count tend to approximate each other on ageing for 7 days. Total count in both butters was predominantly mouldy on ageing. Krishnaswamy and Laxminarayana (1948) observed that desi butter contains more of yeasts and moulds because of its acid environment. Even in the lipolytic count in them, there was a preponderance of moulds after ageing for one week. This clearly indicates that the early deterioration of desi butter and also the ripened cream butter (if the manufacturing methods are unhygienic) is due to lipolytic activity of moulds. In the sweet cream salted butter there is an increase in the total count and yeast and mould count after ageing for 15 days. The lipolytic count was nil both before and after ageing.

Table XVI.

The microbiological and chemical qualities of creamery butter.

	Creamery butter	
	From bakery	Factory butter
Average standard plate count (per ml)	24,000,000	6,900
Average yeast and mould count (per ml)	2,000,000	21
Average lipolytic count (per ml)	100,000	Less than 1
Average coliform count (per ml)	550,000	90
Average serum acidity (percent lactic)	0.17	0.03

Sixteen samples of one oz. (30 grams) packets of butter from bakeries sampled at random were examined for microbiological and chemical qualities, the results of which are presented in Table XVI along with sweet cream salted butter for comparison. It will be seen from the results that the differential counts were very high in butter collected from bakeries than in that from factory. This is of particular importance from public health point of view since this butter is consumed as such without any treatment. This also shows that when manufacturing technique and storage are far from ideal even the creamery butter is likely to be poor in bacteriological quality. The acidity was also higher in butter samples from bakeries.

## CONCLUSION

The microbial quality of different types of butter viz. desi, ripened cream and sweet cream salted butter, was studied with the following results.

(1) Plate count, yeast and mould count, lipolytic count and coliform colonies were maximum in desi butter, moderate in ripened cream butter and least in sweet cream salted butter. High total count in desi butter is accompanied by a high count of spoilage organisms, viz. lipolytic organisms and yeasts and moulds thereby indicating that the manufacturing methods of desi butter is uncontrolled.

(2) As desi butter is heavily contaminated with microflora, the most common deterioration it undergoes, is only rancidity due to lipolysis.

Desi butter deteriorated in 3 days and ripened cream butter in 9 days. Sweet cream salted butter did not develop rancidity even after 15 days. After ageing for 7 days total count and yeast and mould count approximated each other in desi butter, thereby indicating that the acid environment of desi butter is conducive for the proliferation of yeasts and moulds. Also the lipolytic count in desi butter was predominantly mouldy after ageing for 7 days and this leads to the conclusion that the early development of rancidity in desi butter is probably due to moulds.

(3) The quality of butter has certainly an impact on the keeping quality of ghee. The acidity of butter largely contributes to the acidity of ghee which is very well seen from the results. Desi butter has high serum acidity, ripened cream

## SUMMARY

1. The study reveals that plate count is maximum in desi butter with an average of 3,600,000 per ml; moderate in ripened cream butter with an average of 960,000 per ml and least in sweet cream salted butter with an average of 6,900 per ml.
2. High total count is accompanied by a high count of spoilage organisms viz., lipolytic organisms and yeasts and moulds.
3. The differential counts, namely yeast and mould count, lipolytic count and coliform colonies were maximum in desi butter with an average of 510,000, 57,000 and 160,000 per ml respectively; moderate in ripened cream butter with an average of 240,000, 4,700 and 400 per ml respectively and least in sweet cream salted butter with an average of 21, less than 1 and 90 per ml respectively.
4. Desi butter deteriorated earlier than the other two butters. It developed rancidity in 3 days and ripened cream butter in 9 days. Sweet cream salted butter did not deteriorate even after 15 days.
5. Desi butter had an average serum acidity of 0.54 percent lactic acid and ghee made from it had an average acidity of 2.16 percent oleic acid. Ripened cream butter had less serum acidity with an average of 0.17 percent lactic acid and ghee made from it had an average acidity of 0.95 percent oleic acid. Sweet cream salted butter had an average serum acidity of 0.03 percent lactic acid and ghee made from it had an average acidity of 0.26 percent oleic acid.

6. On ageing for 7 days there was an enormous increase in the plate count, lipolytic count and yeast and mould in both desi and ripened cream butters. The lipolytic count in desi butter was predominantly mouldy after ageing for 7 days, thereby indicating that the early development of rancidity in desi butter is probably due to moulds.

7. Creamery butter samples collected at random from bakeries were found to be of poor bacteriological quality.

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