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# Bacteriological studies on butter showing surface taint

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# BACTERIOLOGICAL STUDIES ON BUTTER SHOWING SURFACE TAINT

BY



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## INTRODUCTION

Surface taint is a butter defect that has been encountered so frequently it has been characterized as a definite abnormality and differentiated from other types of deterioration. Undoubtedly different persons do not have exactly the same idea of this defect and there are border-line lots of butter which one judge may consider show surface taint while another does not. The condition may be especially difficult to recognize when the butter also has some other flavor defect. However, the typical defect is easily recognized by butter judges experienced with it.

### Characteristics of Surface Taint Butter

Surface taint is essentially a defect that develops after the butter is made. It makes its first appearance at the surface of the butter and then gradually penetrates to the centre. The odor and flavor of surface taint butter definitely suggest putrefaction. Newly cut surfaces of the butter acquire the taint very rapidly, especially when ex-

posed to room temperature. It ordinarily appears in butter in about ten days after manufacture when stored at a temperature of approximately 5°C. (41°F.). It often appears rather quickly and is sometimes evident in butter only a few days old, especially when the product is exposed to rather high holding temperatures. In other instances reports indicate that butter may be stored for considerable periods and then develop the taint when it warms up during the handling in retail channels. Very often the holding of a small piece of butter at room temperature over night intensifies the odor and flavor so greatly that samples which are questionable before the holding become definite examples.

Surface taint is responsible for considerable economic loss. Butter showing this defect is often unsaleable. In some instances it has been found that working the butter with a small amount of lactic acid improved it so that it was accepted on certain markets.

## Occurrence of Surface Taint Butter

Surface taint was first brought to the attention of the trade in Western Canada in the summer of 1919. Since its appearance in Western Canada, it has been found in other parts of the Dominion. What is essentially the same defect has been discussed in other countries under various names; examples of these are the Limburger cheese flavor of butter

in the United States, the putrid flavor of butter in Denmark and the foetid odor in New Zealand butter. Rather recent reports of butter having a "disagreeable aroma" have come from Australia.

Often churnings of butter that are expected to be of a high quality develop surface taint very rapidly without any reason being evident in the quality of the raw material used or the methods of manufacture employed. It has occurred in one churning then disappeared for several subsequent churnings, only to re-occur.

Most lots of surface taint butter are made from pasteurized cream. In the case of butter made in Canada, the common pasteurizing exposure for the cream is 76.7°C. (170°F.) for ten minutes. As a rule surface taint is found in butter with a low salt content. In very few instances does the salt content of the butter exceed two per cent.

# HISTORICAL

Various investigators have reported data suggesting the cause of surface taint and the related defects in butter.

Gilruth (4) of New Zealand in 1899 developed a foetid odor in butter by inoculating Bacterium fluorescens liquefaciens, which was isolated from water. The odor developed in the butter in thirty-six hours when incubated at 18.3°C. (65°F.). The same odor developed in inoculated butter in one month when it was incubated at a temperature below 0°C. (32°F.). The water supplies of the several factories having this butter defect were considered to be responsible in every case. The practice of liming the wash-water was carried out at one particular factory and, as a result, there was an improvement in the quality of the butter.

In 1900 Eckles (3) studied an outbreak of putrid butter. The butter possessed a strong disagreeable taste with a putrid smell and was considered not saleable at any price for table use. The taste although strong was not as bad as the odor. The investigator found upon examination of this defective butter that when a small portion of it was added to a flask of sterile milk the putrid odor became so bad in a few days time that the flask had to be removed from the laboratory. A bacteriological examination of the putrid butter

showed a large proportion of the organisms present to be of the liquefying type. Four species of micro-organisms capable of producing an objectionable change in milk were isolated. Of the four species isolated two (No. 52 & No. 56) were found to be capable of bringing about the putrid condition in butter when inoculated into cream and churned. Organism No. 52 (bacterium) was considered to bring about the worst condition. Organism No. 56 was found to be Bacterium fluorescens liquefaciens. The creamery having this trouble was advised to reject all raw material suggesting this putrid condition and to clean and sterilize all equipment. The cream for buttermaking was pasteurized and an increased amount of butter culture used. About this same time a dry period which had existed for some time was broken by heavy rains. As a result of some or all these conditions the butter improved and no further trouble was encountered.

The name surface taint was suggested by Marker (9) because the taint is first noticed at the surface of the butter. This investigator has had occasion to examine many churnings of surface taint butter and from the observations made is of the opinion that the reaction of the butter resulting from the neutralization of cream, etc., makes conditions favorable for surface taint development.

Sadler and Vollum (10) studied an outbreak of surface taint butter in Alberta, Canada, and noted that many samples

had an unusually high bacterial count. These investigators found that a spore-forming, milk-digesting micro-organism gave a condition resembling surface taint, particularly when used in low acidity cream (.20%) in combination with a coli type, but failed to give the typical condition. When the same combination of micro-organisms was used in cream of a higher acidity (approx. .30%) there was no evidence of any particular deterioration. A survey of creameries having trouble with surface taint butter showed contamination subsequent to pasteurization to be an important factor. The water supplies used for washing the butter contained many spore-forming, milk-digesting bacteria, and the brine used for the treatment of liners was grossly contaminated with micro-organisms of the coli type. In the case of one creamery, after the necessary precautions had been taken to prevent contamination of the cream and butter subsequent to pasteurization, the trouble ceased.

In a study of surface taint butter, Cordes (2) found it to contain an excessive number of yeasts, molds and bacteria.

The work reported by Macy (8) on surface taint butter showed high counts of yeasts, molds and bacteria. He suggested that the defect was due to a large coccus form in associative action with certain types of yeasts.

Hood and White (6) reported that all samples of surface taint butter examined by them contained large numbers of

yeasts, molds and bacteria. A considerable percentage of the bacteria were of the liquefying type. These investigators failed to produce surface taint with micro-organisms isolated from butter showing this defect, but succeeded in producing it with micro-organisms secured from well water. A bacteriological examination of the water supplies of some of the creameries where surface taint had occurred showed an impure supply in certain cases. In instances where surface taint was produced in butter by liquefying bacteria from well water the organisms were inoculated into pasteurized cream and the cream churned. The taint was evident in from one to two weeks.

The spasmodic occurrence of a defect in Australian butter, described as a "disagreeable aroma", has been studied by Brown (1). The description of this butter defect agrees very closely with that of surface taint. Brown concluded that it is not probable that the defect commences in the manufactured butter, on account of the fact that, after examining many samples of the tainted butter, putrifying bacteria were not constantly found in numbers large enough to cause the trouble. He is of the opinion that the putrescent curdy material which exudes from the glands of the cream storage vats, and the decomposed buttermilk from crevices in the churns, workers etc., become incorporated in the butter during manufacture, and there, in the form of minute particles,

continue to alter, apparently through the action of enzymes and bacterial by-products. The volatile aroma of decomposition characteristic of affected butter is thus produced.

Shutt (11) reported that surface taint was caused by Pseudomonas fluorescens. He isolated this micro-organism from the water supplies of creameries having the trouble. This investigator stated that when pure water was used, or the polluted water pasteurized to 87.7°C. (190°F.), before washing the butter, the trouble ceased. He also recommended as a means of keeping this micro-organism in check, that the acidity of the cream should not be below .35 per cent when churned.

The prevalence of surface taint butter in the provinces of Alberta, Saskatchewan and Manitoba during the years 1928 and 1929 is shown in the following summary which was prepared from data secured by MacKay (7).

PREVALENCE OF SURFACE TAINT BUTTER IN THE PROVINCES OF ALBERTA, SASKATCHEWAN AND MANITOBA IN THE YEARS 1928 AND 1929.

| Province | 1928 | | | 1929 | | | Percentage of Plants Manuf. Surface Taint Butter in 1928 that had a recurrence of Surface Taint in '29 |
|---|---|---|---|---|---|---|---|
| | Total No. of Plants | No. of Plants Manuf. Surface Taint Butter | Percent of Total Lbs. of Butter Examined that showed Surface Taint | Total No. of Plants | No. of Plants Manuf. Surface Taint Butter | Percent of Total Lbs. of Butter Examined that showed Surface Taint | |
| Alta. | 100 | 20 | 3.12 | 95 | 20 | 1.41 | 30.0 |
| Sask. | 82 | 12 | 1.93 | 80 | 6 | 2.52 | 33.3 |
| Man. | 57 | 6 | 1.45 | 58 | 4 | 1.94 | 33.3 |

During the years 1928 and 1929 a total of approximately 140,000 pounds of surface taint butter were detected by dairy produce graders in the provinces of Alberta, Saskatchewan and Manitoba.

## STATEMENT OF THE PROBLEM

Because of the serious losses that have resulted from surface taint in butter, the work herein reported was undertaken.

The investigation was divided into two parts. Part 1 deals with the study of surface taint butter that appeared in regular commercial channels. The study involved the micro-organisms present and the chemical composition and was carried out for the purpose of determining whether or not any unusual bacterial flora could be noted or any unusual change detected in the composition.

Part 2 deals with the production of surface taint in butter by the inoculation into pasteurized cream of surface taint butter or organisms isolated from it. It also deals with the technique used in the isolation of micro-organisms from the original samples of defective butter. Descriptions of micro-organisms that are capable of producing the defect are included.

## METHODS

### Sources of the samples of surface taint butter studied

The surface taint butter used for investigational work was secured from Alberta, Saskatchewan and Manitoba, Canada

and from different sections of the mid-western part of the United States. The ages of the butters examined varied from about ten days to several months. All samples of butter were supposedly made from pasteurized cream and contained salt. Many of the samples examined were manufactured from cream in which butter culture was not used. In a number of instances both the surface and the interior of the butter were examined.

A few samples of normal butter from churnings immediately succeeding and preceding churnings of surface taint butter were obtained from creameries in Canada. This was done in order to make observations on the numbers and types of microorganisms in normal butter produced under the same practical conditions.

Microbiological methods

The microbiological work was gotten under way as soon as possible after receiving the butter samples. In the preparation of the butter for plating, a representative sample was taken from the butter by means of a sterile butter trier or spatula, placed in a sterile container, and melted at a temperature of about 37.5°C.(99.5°F.). After thoroughly mixing the melted butter, the various plates were poured as rapidly as possible. The water blanks were heated to a temperature of about 37.5°C.(99.5°F.).

For the yeast and mold counts, whey agar, adjusted to a reaction of plus 1.0 Fuller's scale, was used. The medium was acidified by adding one cubic centimeter of a sterile one per cent tartaric acid solution to each plate just before pouring in the medium.

For the bacterial counts beef infusion agar, adjusted to a reaction of plus 1.0 Fuller's scale, was used. This medium was chosen because it was desired to use one which would be most favorable for the growth to bacteria found in butter. A milk powder agar was also used to facilitate the detection of proteolytic types. Duplicate plates were prepared for all counts. The plates were incubated at room temperature (approximately 21.1°C. (70°F.)) for five days.

Direct microscopic counts of bacteria were made according to the method of Hammer and Nelson (5).

The cream used for experimental churnings was freshly skimmed from milk of a low bacterial count received at the Dairy Industry Department, Iowa State College. It was pasteurized at 76.7°C. (170°F.) for ten minutes. The churnings were carried out in quart glass jars, in each of which one pint of cream could be churned. The churning was performed by the use of a mechanical shaker. The butter was worked with wooden paddles in small enamel containers. All the equipment employed was of such a nature that it could be sterilized in an autoclave. The salt and wash water were

also sterilized. The butter was stored in either sterile parchment paper or Petri dishes at temperatures of 15.5°C. (60°F.) and 5.5°C. (42°F.). The butters were examined daily for any change in flavor and odor.

Chemical methods

Acidity determinations were made by weighing 10 grams of butter and melting it over a flame. To the melted butter 35 c.c. of neutral anhydrous ether were added to dissolve the fat. After thoroughly mixing, 10 c.c. of neutral ethyl alcohol were added and the mixing repeated. This mixture was then titrated with tenth normal NaOH, using phenolphthalein as an indicator. The results are reported as the number of c.c. of tenth normal NaOH required for neutralization.

In studying proteolysis in the surface and in the interior portions of surface taint butter, total, soluble and amino nitrogen were determined. A 180 gram portion of butter was melted in a beaker by heating in a water bath, the temperature of which was approximately 55°C. (131°F.). After the melted fat was satisfactorily separated, as much as possible of it was decanted, care being taken to retain all the non-fatty material. The contents of the beaker were then transferred to a separatory funnel, and, by repeated washing with

warm water the non-fatty material was separated from the remaining fat and collected in a 300 c.c. volumetric flask. Additional water was then added to bring the contents of the flask up to the 300 c.c. graduation.

For total nitrogen determinations, 50 c.c. of the non-fatty material were transferred to a 500 c.c. Kjeldahl flask and digested with 25 c.c. of concentrated $H_2SO_4$, approximately 5 grams of $Na_2SO_4$ and a small piece of copper. The distillates were collected in fifth normal $H_2SO_4$ and back titrations made with tenth normal NaOH, using monosodium alizarine-sulphonate as an indicator.

The remaining 200 c.c. of the non-fatty material (two 50 c. c. portions having been used) were employed for soluble and amino nitrogen determinations. These were divided into 100 c.c. portions and slightly heated. The insoluble constituents were flocculated by the addition of approximately 3 c.c. of a tenth molar solution of $AlCl_3$, added in one cubic centimeter portions, with vigorous shaking after each addition. The portions were allowed to stand for a few minutes, then cooled and filtered through paper. The soluble nitrogen was determined by the Kjeldahl procedure as already outlined, except that 25 c.c. portions were used, and the amino nitrogen was determined by the Van Slyke method, using 10 c.c. of the filtrate.

The lactose was determined by the gravimetric method as outlined in the Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, using the serum from 25 grams of butter.

In determining the reaction of the material distilled from butter a 250 gram portion of the butter was distilled with steam, after the addition of 50 c.c. of boiled distilled water. Distilled water was used in the steam can and was allowed to boil for some time before connecting and starting the distillation in order to drive off the CO2 present. The distilling was continued until ten consecutive portions of 50 c.c. each were secured. In determining the relative acidity of the different portions by titration, 25 c.c. of each of the portions of distillate were titrated with twentieth normal NaOH, or twentieth normal H2SO4 depending upon the reaction of the distillate, using mono-sodium-alizarine-sulphonate as an indicator. The results obtained were expressed as the number of cubic centimeters of twentieth normal NaOH or twentieth normal H2SO4 required for the neutralization of the 25 c.c. Colorimetric determinations were made by taking 5 c.c. of each portion of a distillate and after adjusting the temperature between 30°C. (86°F.) and 40°C. (104°F.) adding ten drops of brom thymol blue indicator to each portion. The samples were then shaken and the color noted.

# PART 1

## STUDIES ON SURFACE TAINT BUTTER THAT APPEARED IN REGULAR COMMERCIAL CHANNELS.

### MICROBIOLOGICAL

#### Numbers of bacteria

Samples of surface taint butter were examined by the plate method for the total number of bacteria contained. With some of the samples both surface and interior portions were studied, while with others the amount available was so small that this could not be done. Table I shows the numbers of bacteria at the surface and in the interior of 20 samples of butter, while table II shows the numbers of bacteria in 15 samples where a division into surface and interior portions was not possible.

The data given show that the surface taint butter commonly contained large numbers of organisms. The surface of the butter usually showed a count much higher than the interior. The high counts are in agreement with the findings of various investigators. It should be noted, however, that this condition is not peculiar to surface taint butter but also occurs with butter showing various flavor and aroma defects.

Also, normal butter may often show bacterial counts that are extremely high. This is particularly true in the

TABLE I. Total bacteria in surface taint butter by the plate method.

| Sample No. | Bacteria per c.c. of butter | |
|---|---|---|
| | Surface | Interior |
| 1 | 303,000,000 | 14,000,000 |
| 2 | 36,000,000 | 22,400,000 |
| 3 | 137,500,000 | 18,000,000 |
| 4 | 20,500,000 | 6,500,000 |
| 5 | 20,000,000 | 18,200,000 |
| 6 | 65,200,000 | 29,800,000 |
| 7 | 5,850,000 | 1,650,000 |
| 8 | 72,000,000 | 950,000 |
| 9 | 18,900,000 | 11,900,000 |
| 10 | 1,030,000 | 565,000 |
| 11 | 28,500,000 | 2,450,000 |
| 12 | 129,000,000 | 25,000,000 |
| 13 | 16,000,000 | 6,000,000 |
| 14 | 19,000,000 | 7,350,000 |
| 15 | 69,500,000 | 29,000,000 |
| 16 | 1,940,000 | 148,000 |
| 17 | 5,600,000 | 3,200,000 |
| 18 | 9,800,000 | 2,000,000 |
| 19 | 1,840,000 | 2,400,000 |
| 20 | 2,100,000 | 50,000 |

TABLE II. Bacteria in surface taint butter by the Plate method.

| Sample No. | Bacteria per c.c. of Butter |
|---|---|
| 21 | 9,250,000 |
| 22 | 13,000,000 |
| 23 | 177,500,000 |
| 24 | 11,000 |
| 25 | 39,000,000 |
| 26 | 445,000 |
| 27 | 2,500,000 |
| 28 | 37,000,000 |
| 29 | 5,200,000 |
| 30 | 1,500,000 |
| 31 | 30,000,000 |
| 32 | 18,000,000 |
| 33 | 20,000,000 |
| 34 | 14,000,000 |
| 35 | 600,000 |

case of butter manufactured from cream to which butter culture has been added. An examination of several samples of normal butter produced by creameries from which surface taint samples were secured gave the following bacterial counts: 28,000,000; 17,500,000; 20,000; 54,000,000; 4,900,000. The above counts are typical of many samples of high scoring butter and demonstrate the fact that the kinds rather than the numbers of bacteria are often of the greater importance.

From the results it is evident that high bacterial counts are usually secured on surface taint butter. The surfaces of the butter commonly contain greater numbers of bacteria than the interior. The data further suggest that the numbers of bacteria are greatly in excess of what the bacterial count should be in butter manufactured under careful conditions.

Numbers of yeasts and molds

Samples of surface taint butter were examined by the plate method for the numbers of yeasts and molds. Table III presents the results obtained.

An analysis of the data shows that the numbers of yeasts were regularly high while the numbers of molds were generally high. In most instances the surfaces of the defective butters showed higher yeast and mold counts than the interior. In several instances molds were absent in both the surface and interior of the butter. The common milk mold, Oidium lactis,

TABLE III. Yeasts and molds in surface taint butter by the plate method.

| Sample | Yeasts per c.c. of butter | | Molds per c.c. of butter | |
|---|---|---|---|---|
| | Surface | Interior | Surface | Interior |
| 1 | 108,500 | 770 | 0 | 0 |
| 2 | 3,000,000 | 965 | 45 | 20 |
| 3 | 2,000 | 940 | 70 | 0 |
| 4 | 24,000 | 62,000 | 35 | 20 |
| 5 | 16,000 | 4,400 | 20 | 10 |
| 6 | 2,750 | 2,400 | 75 | 10 |
| 7 | 70,600 | 54,800 | 50 | 0 |
| 8 | 43,200 | 190 | 10 | 5 |
| 9 | 97,500 | 47,000 | 1,000 | 0 |
| 10 | 80 | 210 | 10 | 0 |
| 11 | 170 | 50 | 0 | 0 |
| 12 | 70,000 | 9,000 | 1,000 | 13,000 |
| 13 | 464,000 | 200,000 | 28,000 | 0 |
| 14 | 200,000 | 153,000 | 100 | 0 |
| 15 | 2,000 | 450 | 5 | 0 |
| 16 | 35,000 | 21,000 | 0 | 0 |
| 17 | 1,400 | 1,100 | 10 | 20 |
| 18 | 4,400 | 2,600 | 10 | 0 |
| 19 | 104,000 | 80 | 10 | 0 |
| 20 | 320,000 | 23,000 | 10 | 10 |

was the one most frequently found. Occasionally species of the genus Penicillium were present in fairly large numbers.

The results indicate that the numbers of yeasts and molds in surface taint butter are excessive in most instances.

Types of bacteria

A study of the types of bacteria present at the surface and in the interior of surface taint butter was made, because the nature of the defect suggested that the causative micro-organism or micro-organisms would be present in greater numbers at the surface of the butter. This study was carried out by picking into litmus milk 25 contiguous colonies from representative areas on the plates. These milk cultures were incubated at room temperature for 14 days and then classified, according to the change produced, into the following groups: acid coagulators, acid non-coagulators, proteolyzers; alkali formers and inert. The results are reported on a percentage basis in table IV.

The data show that in general the types of bacteria present in the butter were the same at the surface as in the interior. A large percentage of the bacteria were classified as acid non-coagulating or inert and these did not produce an objectionable odor in milk. A small percentage of acid coagulating bacteria is to be expected since the butters

TABLE IV. Types of bacteria present in surface taint butter.

| Sample | | Percentage | | | | |
|---|---|---|---|---|---|---|
| | | Acid | | | | |
| | | Coagulators | Non-coagulators | Proteolysers | Alkali Formers | Inert |
| 1 | Surface | 8.0 | 64.0 | | | 28.0 |
| | Interior | | 64.0 | 4.0 | | 32.0 |
| 2 | Surface | 4.0 | 56.0 | | 4.0 | 36.0 |
| | Interior | 4.0 | 52.0 | | | 44.0 |
| 3 | Surface | | 80.0 | | | 20.0 |
| | Interior | | 88.0 | | | 12.0 |
| 4 | Surface | | 44.0 | | 24.0 | 32.0 |
| | Interior | | 68.0 | 4.0 | 8.0 | 20.0 |
| 5 | Surface | | 52.0 | 28.0 | 4.0 | 16.0 |
| | Interior | | 64.0 | 20.0 | | 16.0 |
| 6 | Surface | | 40.0 | 4.0 | 4.0 | 52.0 |
| | Interior | | 64.0 | 8.0 | 4.0 | 24.0 |
| 7 | Surface | | 68.0 | | | 32.0 |
| | Interior | | 76.0 | | | 24.0 |
| 8 | Surface | | 88.0 | | | 12.0 |
| | Interior | | 95.0 | 5.0 | | |
| 9 | Surface | | 24.0 | | 16.0 | 60.0 |
| | Interior | | 24.0 | 4.0 | 8.0 | 64.0 |
| 10 | Surface | | 32.0 | | 4.0 | 64.0 |
| 11 | Surface | 28.0 | 24.0 | 12.0 | 20.0 | 16.0 |
| 12 | Surface | 8.0 | 56.0 | 16.0 | 12.0 | 8.0 |
| | Interior | 40.0 | 40.0 | 20.0 | | |
| 21 | | 56.0 | | 4.0 | 16.0 | 24.0 |
| 22 | | 48.0 | 20.0 | | 4.0 | 28.0 |
| 23 | | 40.0 | 8.0 | 36.0 | 4.0 | 12.0 |

examined were made from cream in which butter culture was not used. The comparatively small percentages of bacteria capable of proteolyzing milk are surprising since the nature of surface taint suggests protein decomposition.

On the basis of morphology, micrococci were especially conspicuous, although rods, both spore-forming and non-spore-forming, and streptococci were frequently encountered in large numbers. The predominating type was not always the same. The results suggest that if a micro-organism capable of growth on beef infusion agar is responsible for surface taint it must be present in much smaller numbers than other less objectionable types. The proportion of rods present in surface taint butter, as revealed by direct microscopic observations, was greater than the proportion on the plates poured with beef infusion agar. This suggests that certain types of rods in the butter were not growing on the medium used.

In general, the types of bacteria found in surface taint butter are not unusual. The bacterial flora of normal butters show a distribution of types not unlike that of surface taint butter. The large percentage of acid non-coagulating or inert types of bacteria is the common findings in most lots of surface taint butter. The results, on the whole, indicate that types of bacteria capable of bringing about proteolysis are either present in very small numbers, or are not growing on the artificial medium used.

## CHEMICAL

### Acidity in surface taint butter

Acidity determinations were made on surface and interior portions of surface taint butter. The results are given in table V.

In general, the data show that there is no regular difference between the surface and interior of the butter. However, the figures are significant in that they show the very low acidity that existed in the defective butter. This low acidity is, in general, favorable for the growth of a very large percentage of the bacteria that are commonly found in butter. The sensitiveness of most organisms to acidity is strikingly evident when butter manufactured from cream to which butter culture has been added is examined for types of bacteria.

From the data it is evident that there is no general difference in the acidity, between the surface and interior of the butter, that could be attributed to the development of surface taint.

### Proteolysis in surface taint butter

Proteolysis was determined in surface taint butter because the nature of the defect suggests protein decomposition. Casein is a constituent of butter whose decomposition

TABLE V. Acidity determinations on surface taint butter.

| Sample No. | Acidity per 10 gms Butter | |
|---|---|---|
| | Surface c.c. N/10 NaOH | Interior c.c. N/10 NaOH |
| 1 | 1.50 | 1.50 |
| 2 | 1.70 | 1.70 |
| 3 | 1.07 | 1.35 |
| 4 | 1.05 | 1.17 |
| 5 | 1.00 | 1.07 |
| 6 | 1.10 | 1.10 |
| 7 | 1.45 | 1.40 |
| 8 | 1.47 | 1.45 |
| 9 | 2.15 | 1.40 |

would be expected to result in objectionable conditions. It is present in amounts sufficient to support extensive development of micro-organisms. In order to secure information on the extent of proteolysis in surface taint butter, surface and interior portions of several samples were examined for the total, soluble and amino nitrogen. Table VI presents the results secured.

As would be expected, the total nitrogen at the surface and in the interior of the butter did not vary appreciably. There is a general difference in soluble nitrogen between the surface and interior of the butter, with the surface showing a larger amount. The same relationship held with the amino nitrogen. These evidences of more active proteolysis at the surface of the butter further substantiate the possibility of protein decomposition being responsible for the surface taint defect. One must bear in mind that even the slightest change in the nature of the nitrogenous compounds might be sufficient to bring about a very marked change in the odor or flavor of the butter. A hydrolysis of the protein, in which the protein is split up into amino acids, and the further decomposition of these acids with the formation of ammonia and various foul-smelling substances, could easily bring about such a condition as surface taint without a very marked change being noted in the composition of the butter.

TABLE VI. Proteolysis in surface taint butter.

| Sample No. | Nitrogen per 10 c.c. Filtrate | | |
|---|---|---|---|
| | Total mgms. | Soluble mgms. | Amino mgms. |
| 1 Surface | 3.374 | 2.352 | 0.795 |
| Interior | 3.346 | 1.664 | 0.740 |
| 2 Surface | 3.682 | 2.128 | 0.658 |
| Interior | 3.688 | 1.440 | 0.575 |
| 3 Surface | 3.324 | 1.736 | 0.575 |
| Interior | 3.352 | 1.400 | 0.603 |
| 4 Surface | 2.134 | 0.924 | 0.461 |
| Interior | 2.134 | 0.684 | 0.420 |
| 5 Surface | 2.772 | 1.204 | 0.598 |
| Interior | 2.786 | 0.964 | 0.489 |
| 6 Surface | 2.926 | 0.940 | 0.661 |
| Interior | 2.890 | 0.700 | 0.441 |
| 7 Surface | 3.612 | 0.684 | 0.466 |
| Interior | 3.444 | 0.560 | 0.453 |
| 8 Surface | 3.402 | 1.820 | 0.644 |
| Interior | 3.150 | 1.720 | 0.613 |

It is evident that the differences in the soluble and amino values between the surface and interior of the butter indicate proteolysis. Apparently micro-organisms with the ability to proteolyze are, or have been, present in surface taint butter and are influential in bringing about this defect.

Lactose in surface taint butter

A study of the lactose present at the surface and in the interior of surface taint butter was undertaken to determine whether any appreciable change could be detected. Lactose is one of the constituents of dairy products that is usually the first to undergo change, as there are many organisms found in dairy products that readily attack it. Only a small amount of lactose is found in butter since the washing of the butter during manufacture necessitates its removal. The results of the lactose determinations are given in table VII.

An examination of the data shows that in most cases there is a slightly lower percentage of lactose at the surface than at the interior of the defective butter. As the differences are very small it is problematical as to whether any significance can be attached to them.

The results, on the whole, indicate that the slight differences in amounts of lactose at the surface and in the interior of the butter are insufficient to attach any signifi-

TABLE VII. Lactose determinations on surface taint butter.

| Sample No. | Percent Lactose | |
|---|---|---|
| | Surface | Interior |
| 1 | 0.2940 | 0.5328 |
| 2 | 0.1556 | 0.1760 |
| 3 | 0.4050 | 0.4126 |
| 4 | 0.0928 | 0.1274 |
| 5 | 0.0700 | 0.1276 |
| 6 | 0.1080 | 0.1820 |
| 7 | 0.3848 | 0.3888 |
| 8 | 0.3460 | 0.4052 |
| 9 | 0.3072 | 0.3674 |

cance to them, since the acidities, already discussed, do not show any appreciable change.

Steam distillations of surface taint and normal butters

A number of samples of surface taint and normal butter, secured from Canada, were steam distilled in order to compare the reaction of the material distilled from defective butter with that from normal butter. The samples of normal butter were obtained from churnings immediately preceding or succeeding the churnings of surface taint butter. The distillates collected from surface taint butters showed a very pronounced surface taint odor. Even after storing the distillates for several days the odor could be readily detected. The intensity of the odor was very noticeable when comparisons were made between the different portions collected during the process of distillation, the first few portions collected showing a pronounced odor, while the latter portions showed but very little or no odor. Titrations were made on 25 c.c. aliquots of the ten consecutive portions of each distillate,* using twentieth normal acid or alkali as required and monosodium-alizarine-sulphonate as an indicator. The results obtained are shown in table VIII. The reaction of the distillates were also determined colorimetrically, using brom thymol blue to indicate whether the material was acid or alkaline. Portions of the distillates that were below pH 7 were considered

* The distillation procedure is given under "Methods".

TABLE VIII. Titration values on distillates from surface taint and normal butter.

| Aliquot | c.c. of n/20 alkali or acid per 25 c.c of distillate | | | | |
|---|---|---|---|---|---|
| | Comparison 1 | | Comparison 2 | | |
| | Normal Butter | Surface Taint Butter | Normal Butter (High Grade) | Surface Taint Butter | Normal Butter (Low Grade) |
| 1 | - 0.27 | - 1.35 | - 0.30 | - 0.37 | + 0.32 |
| 2 | - 0.22 | - 0.50 | - 0.27 | - 0.27 | + 0.30 |
| 3 | - 0.20 | - 0.25 | - 0.22 | - 0.20 | + 0.27 |
| 4 | - 0.20 | - 0.15 | - 0.22 | - 0.15 | + 0.25 |
| 5 | - 0.17 | - 0.15 | - 0.20 | - 0.12 | + 0.22 |
| 6 | - 0.15 | - 0.10 | - 0.17 | - 0.11 | + 0.20 |
| 7 | - 0.13 | - 0.12 | - 0.17 | - 0.11 | + 0.20 |
| 8 | - 0.12 | - 0.10 | - 0.17 | - 0.10 | + 0.20 |
| 9 | - 0.11 | - 0.10 | - 0.15 | - 0.07 | + 0.20 |
| 10 | - 0.11 | - 0.10 | - 0.12 | - 0.07 | + 0.20 |

- = alkaline

+ = acid

acid, while those portions that were above pH 7 were considered alkaline. The data are shown in table IX.

The data presented in table VIII show that in comparison 1 the first portion of distillate collected from the surface taint sample was definitely more alkaline than the first portion of distillate collected from the normal sample. In comparison 2 there is no appreciable difference between the distillates collected from the normal butter and the distillates collected from the surface taint butter. The normal low grade butter was quite different from the other two in that all the distillates were definitely acid. Tests reported in table IX show that in general the alkaline reaction persisted through more aliquots of distillate in the case of surface taint butter than in the case of normal butter.

The results obtained on the few samples examined indicate that distillates secured from surface taint butter by steam distillation have a greater alkalinity than those from normal butter. This reaction relationship may explain why the addition of some mild acid, such as lactic or boric acid, to surface taint butter in the process of reworking tends to overcome the defect. The change in reaction of the butter makes conditions more or less unfavorable for the growth of organisms and, accordingly, may prevent the re-development of the defect.

TABLE IX. Colorimetric test on distillates from surface taint and normal butter.

| Aliquot | Comparison 1 | | Comparison 2 | | |
|---|---|---|---|---|---|
| | Normal Butter | Surface Taint Butter | Normal Butter (High Grade) | Surface Taint Butter | Normal Butter (Low Grade) |
| 1 | - | - | - | - | + |
| 2 | - | - | + | - | + |
| 3 | - | - | + | - | + |
| 4 | + | - | + | - | + |
| 5 | + | + | + | + | + |
| 6 | + | + | + | + | + |
| 7 | + | + | + | + | + |
| 8 | + | + | + | + | + |
| 9 | + | + | + | + | + |
| 10 | + | + | + | + | + |

- = alkaline

+ = acid

PART 2

## THE PRODUCTION OF THE SURFACE TAINT CONDITION IN BUTTER

### By the use of commercial surface taint butter as inoculating material

The initial attempts to produce surface taint involved the inoculation of surface taint butter into normal butter. This procedure was carried out by taking a small amount of the defective material and thoroughly working it into the normal product by the use of a butter spade. Both salted and unsalted butter were inoculated in this manner and incubated at 15.5°C. (60°F.) and 5.5°C. (42°F.). The samples of butter held at the different temperatures were examined periodically for changes in flavor and odor. The results obtained with this method were regularly unsuccessful.

The addition of surface taint butter to the cream shortly before churning was tried in an endeavor to produce the taint. Cream pasteurized at a temperature of 76.7°C. (170°F.) for ten minutes was inoculated with a small amount of melted surface taint butter. Care was exercised in the melting of the defective butter so that the temperature would not exceed 40°C. (104°F.). This precaution was taken in order to prevent any possibility of organisms being killed by the heat, and also to prevent a complete separation of the butter fat and serum. The melted butter was carefully dis-

tributed over the surface of the cream and then the cream thoroughly shaken. If the melted butter is merely dumped into the cold cream carelessly it will solidify immediately and there will not be a satisfactory distribution of the micro-organisms added. The cream was churned either immediately or after holding over-night at about 15.5°C. (60°F.). The resulting butter was washed twice with sterile water. The amount of water used in the first washing was just sufficient to rinse the butter granules while the second washing consisted of sufficient water to fill the churn half full with thorough agitation. After draining off the wash-water the butter granules were transferred to a sterile container for working. Both salted and unsalted butter were prepared and held at 15.5°C. (60°F.) and 5.5°C. (42°F.). In the case of salted butter, sufficient sterile salt was added so as to have approximately one to one and one-half per cent in the finished butter.

The experimental butter resulting from many of these trials quickly developed a condition considered to be surface taint. A pronounced taint in the butter required from one to three days at 15.5°C. (60°F.) and from seven to ten days at 5.5°C. (42°F.) for its development. The aforementioned procedure of reproducing surface taint was successful in a considerable number of cases with samples of commercial surface taint butter secured from different parts of Canada and the United States.

Experimentally produced surface taint butter also quite regularly caused the defect in butter made from pasteurized cream. As already described, a small portion of the melted butter was carefully added to the cream, the cream churned and the resulting butter stored and examined periodically. In this way the defect was sometimes produced down through a series of several churnings. Eventually, however, some other flavor or odor would over-shadow the surface taint and become the predominating defect in the subsequent lots. One of the most common defects that over-shadowed the surface taint was rancidity. On holding surface taint butter that tended to be rancid it acquired a strong odor and flavor and became extremely rancid.

Occasionally samples of butter were received that were diagnosed by the parties sending them as surface taint. Upon examining these butters the odors and flavors suggested poor raw material, rather than odors and flavors that were developed after the butters were manufactured. Instead of the characteristic surface taint odor and flavor, a stale, yeasty, cheesy odor and flavor suggestive of stale fermented cream could be readily detected. Attempts to reproduce surface taint with these supposedly surface taint butters were unsuccessful. These failures to produce surface taint, or any other conspicuous odor or flavor, substantiate the fact that the defect in the butter was originally due to the poor

quality of the raw material used. A bacteriological examination of these butters very often showed only a few thousand bacteria per cubic centimeter, indicating that deterioration due to growth after the butter was manufactured was not a factor in the poor quality of the finished product.

By holding high grade butter with little or no salt at room temperature

In studying the keeping quality of a large number of samples of high grade butter, secured from different sources and stored at room temperature for seven days, it was found that quite a number of them developed a defect that was considered to be surface taint. The characteristic putrid odor and flavor developed was very conspicuous and was without a doubt the same as the so-called surface taint. In most cases these lots of butter were of a low salt content or unsalted. Butter produced by churning pasteurized cream that had been inoculated with a small portion of one of the defective samples, regularly developed surface taint in a few days when stored at the temperatures that were employed in the trials made with commercial surface taint butter. As in the former instances, the defect could be produced down through a series of churnings.

Microscopic examinations of experimentally produced surface taint butter

Microscopic examinations of experimentally produced surface taint butter regularly showed large numbers of organisms. In many instances the numbers were half a billion and over. These excessive numbers of organisms in experimentally produced surface taint butter are in agreement with what was found in microscopic examinations of commercial surface taint butter. The enormous numbers of organisms in the butter strikingly suggest the relationship of organisms to the surface taint condition. It must be admitted, however, that often samples of high grade butter may show a very high bacterial count. This is due to many of the bacteria that have been killed by the process of pasteurizing the cream appearing in the counts, and to the growth of certain types that do not seriously affect the flavor and odor.

Microscopic examinations of experimentally produced surface taint butter, at the time the defect was first detected, showed a large proportion of long, slender rods. The association of rod-shaped bacteria with surface taint butter appears to be quite constant. The samples of commercial surface taint butter and the high quality butter that developed surface taint when held under conditions favorable for the growth of organisms also showed a predominance of rod-shaped bacteria when examined microscopically.

## Significance of the experimental production of surface taint

The ability of surface taint butter to reproduce the defect down through a series of churnings, and the development of surface taint by holding at room temperature samples of high grade butter having a low salt content suggest the biologic nature of the defect. Apparently organisms capable of producing surface taint may be in butter and yet not bring about the defect because of unsatisfactory conditions. It is only after the introduction of the proper kinds of organisms to the cream and the handling of the butter so as to allow these organisms a chance to develop that surface taint occurs. The results obtained also indicate that comparatively unobjectionable types of organisms may be present in larger numbers than those responsible for surface taint.

## The production of surface taint with pure cultures of organisms

Many of the bacteria found in the commercial samples of surface taint butter were classified as acid non-coagulating or inert. Although the odors, flavors and general changes produced in milk by these common types were not suggestive of the surface taint condition, a considerable number of the organisms were studied in an endeavor to reproduce the taint in butter. They were used singly and in various combinations.

The first trials were made by inoculating normal butter with little or not salt and were regularly unsuccessful. Since the method of inoculating the cream had been so satisfactory in producing the condition when defective butter was used as the inoculating material, this procedure was employed in the later trials with the pure cultures. Many of the easily isolated types of bacteria from samples of surface taint butter were inoculated ino pasteurized cream, singly and in various combinations, and butter made in an endeavor to produce surface taint. The butters were stored at 15.5°C. (60°F.) and 5.5°C. (42°F.) and examined periodically for surface taint development. A large percentage of the samples of butter kept remarkedly well and did not develop any particular off flavor or odor. Those butters that did develop off flavors and odors did not show a condition even remotely resembling surface taint.

Since the types of bacteria which are numerous in surface taint butter and easily isolated from it failed to produce the defect, an endeavor was made to isolate types present in much smaller numbers and to study their influence on butter. In selecting cultures to be studied in detail it seemed advisable to take the odor produced in milk as a basis. A large number of samples of commercial surface taint butter were examined in this endeavor. The common procedure was to pick colonies from beef infusion agar plates into

litmus milk, incubate the milk cultures for 14 days at room temperature and then make observations on the changes taken place.

Eventually an organism, tentatively called A, capable of causing surface taint was isolated from a sample of commercial surface taint butter. This was accomplished in the usual way by plating the defective butter on beef infusion agar and picking colonies into litmus milk after several days incubation at room temperature. When a culture of this organism was inoculated into pasteurized cream and the cream churned the resulting butter developed surface taint in a few days when stored at 15.5°C. (60°F.) and in from seven to ten days at 5.5°C. (42°F.) The oder developed in butter by this micro-organism is very typical of surface taint. The organism was apparently present in comparatively small numbers as judged by beef infusion agar plates.

The results obtained with organism A led to the examination of other lots of surface taint butter for its presence. Approximately 50 samples of commercial surface taint butter were examined; many of these were from Canada while a few were from the United States. The usual procedure of plating the surface taint butter on beef infusion agar and picking colonies into litmus milk after several days incubation at room temperature, failed to yield organism A from these samples. The high dilutions necessary in order to obtain agar plates

that would not be too crowded for picking colonies naturally lessened the chances of isolating the organism.

The failure of the direct plating procedure to yield organism A from all except the one sample of surface taint butter suggested the possibility of other methods of isolation. The outstanding ability of this organism to rapidly reduce litmus milk was useful in detecting its presence. After many trials an enrichment scheme proved successful in yielding organism A from five additional samples of commercial surface taint butter. This was accomplished by inoculating the defective butter into litmus milk and incubating at 5.5°C. (42°F.) until the litmus was reduced, then plating on beef infusion agar and picking colonies into litmus milk after several days incubation at room temperature. The incubation of the litmus milk at 5.5°C. (42°F.) did not always prevent the development of acid producing types and, as a result, a reduction of the litmus milk was often shown when organism A was not present.

The six samples of surface taint butter from which organism A was isolated were from six creameries, four in Canada and two in the United States.

Although organism A was present in the six samples of butter in comparatively small numbers it could always be found when later attempts were made to isolate it. Its out-

standing ability to reduce litmus milk in a few hours makes it rather easy to recognize. It does not seem probable that this organism died out completely from some samples of surface taint butter since surface taint butter produced experimentally yielded the organism after several months storage at 5.5°C. (42°F.). The comparatively small numbers in which organism A was found in surface taint butter made its relationship to the defect open to question even if it was capable of producing a typical surface taint condition. Accordingly, experimental churnings were made by inoculating the organism into pasteurized cream and determining the numbers of organisms in the butter as soon as the defect developed; the counts were made by the method of plating on beef infusion agar and incubating for five days at room temperature. Representative data are presented in table X.

TABLE X. Numbers of bacteria at the time of surface taint development in experimental butters made with a pure culture of organism A.

| Age of Butters in Days | Trials 1 | 2 | 3 | Flavor and odor |
|---|---|---|---|---|
| 0 | 3,000 | 10,000 | 10,000 | Normal |
| 2 | 42,000 | 180,000 | 900,000 | Surface Taint |
| 6 | 440,000 | 900,000 | 290,000 | Surface Taint |

The results show that surface taint may be present in butter when the bacterial count is only a few hundred thousand per cubic centimeter or even less than one hundred thousand. An examination of the colonies present on the agar plates showed practically a pure culture of organism A. These facts strikingly demonstrate that comparatively few organisms of type A are required in order to bring about the surface taint condition.

The failure to find organism A in all samples of surface taint butter, even when examined by a number of procedures, led to the search for other types capable of causing the condition. The method of direct plating, using beef infusion agar and differential media, such as milk powder agar, was used. After incubating the plates for a few days at room temperature representative colonies were picked into litmus milk. In another method plates were poured with beef infusion agar and, after they had been allowed to solidify, the surface of one was smeared with a small portion of the defective butter by the aid of a sterile bent glass rod. The butter adhering to the glass rod was then transferred to the next plate and so on through at least three plates. After incubating for a few days at room temperature representative colonies were picked into litmus milk. By this method it was hoped to reduce the danger of diluting out the causative organism.

A method of inoculating the surface taint butter into milk and making a series of dilutions with the object of di-

luting out the organisms that were unimportant was attempted. The oder produced in the milk and the type of colonies picked from beef infusion agar plates were considered in determining the results.

A series of counts were made on commercial and experimental surface taint butter by the direct and plate methods. The data presented in table XI are representative of the results obtained. The counts were regularly higher by the direct method than by the plate method. In some cases the differences are very large while in other cases they are no greater than would be expected, considering the differences that occur with milk. Of course, many of the bacteria as revealed by the direct method may have been dead. The differences between the results of the two methods suggested the possibility of anaerobic bacteria being in the butter and not growing on the plates. While anaerobes did not seem a probable cause of surface taint because the condition develops first at the surface of the butter, there is the possibility that anaerobic bacteria growing in the interior of the butter form products which are changed on exposure to air so that the characteristic oder results.

Different methods of growing anaerobic bacteria were undertaken in an effort to isolate organisms capable of causing surface taint. Test tubes of sterile litmus milk, which had been boiled to drive off any oxygen present and then im-

TABLE XI. A comparison of the plate count and direct count of samples of butter showing surface taint.

| Sample No. | Bacteria per c.c. of butter | |
|---|---|---|
| | Plate Count | Direct Count |
| 36 | 143,000,000 | 530,000,000* |
| 37 | 20,000,000 | 500,000,000 |
| 38 | 18,000,000 | 270,000,000 |
| 39 | 30,000,000 | 213,000,000 |
| 40 | 600,000 | 530,000,000 |
| 41 | 30,000,000 | 105,000,000 |
| 42 | 13,000,000 | 800,000,000 |
| 43 | 126,000,000 | 320,000,000 |
| 44 | 144,000,000 | 430,000,000 |
| 45 | 14,000,000 | 530,000,000 |
| 46 | 9,000,000 | 270,000,000 |
| 47 | 6,800,000 | 320,000,000 |
| 48 | 31,000,000 | 430,000,000 |

*Count estimated on samples with 500,000,000 bacteria or over.

mediately cooled by immersing in ice-water, were inoculated with surface taint butter, sealed by pouring about one-half inch of sterile vaseline on its surface and then incubated at room temperature. A medium consisting of sterile sheep brains was also inoculated with surface taint butter and incubated at room temperature. These methods of providing anaerobic conditions changed the flora that was originally present in the surface taint butter at the time of inoculation. The predominating types present did not bring about any marked change in litmus milk, and were inert with regard to bringing about any odor suggestive of surface taint when inoculated into pasteurized cream and the cream churned.

Experiments were undertaken to determine the effects of changes in atmospheric conditions by partial displacement or full displacement of the air by carbon dioxide. Plates inoculated with surface taint butter were poured with beef infusion agar and place in Novy jars and the lids sealed with vaseline. An apparatus was constructed so that the air displaced by carbon dioxide could be measured. The plates were incubated at room temperature for five days and then examined. In the case where all the air was displaced by carbon dioxide there was little or no growth of organisms, but just as soon as such plates were given an air supply the organisms grew. When there was partial displacement of the air a considerable number of organisms found conditions favorable for growth.

From a number of samples of surface taint butter, organisms, other than A, were found which would produce the condition. These were not all of the same type and were classified into three groups, making a total of four groups including A.

In all, cultures of organisms which would produce surface taint were isolated from 17 samples of commercial surface taint butter. Six of the samples were from Canada and 11 from the United States. Table XII contains a summary of the different types of organisms isolated and their sources. All the cultures isolated grew well on beef infusion agar so that their presence in small numbers is the only excuse why they should be missed when surface taint butter is plated. Most of the organisms were gotten by the usual method of direct plating of surface taint butter on beef infusion agar and picking colonies after incubation for five days at room temperature, or by smearing the surface taint butter on beef infusion agar plates that had been previously poured and allowed to solidify and then picking colonies after incubation for a few days at room temperature. A few of the cultures were isolated by the enrichment scheme of inoculating the surface taint butter into litmus milk, incubating at 5.5°C. (42°F.) for several days, then plating the milk on beef infusion agar and picking colonies after incubating a few days at room temperature.

TABLE XII. Types of organisms capable of producing surface taint and their sources.

| Type | Total No. of Cultures | Source: Canada — No. of Cultures | Canada — No. of Creameries from which the type was found | United States — No. of Cultures | United States — No. of Creameries from which the type was found |
|---|---|---|---|---|---|
| A | 6 | 4 | 4 | 2 | 2 |
| B | 6 | 1 | 1 | 5 | 4 |
| C | 1 | 0 | 0 | 1 | 1 |
| D | 4 | 1 | 1 | 3 | 2 |

In general, the methods of direct plating or smearing the butter on solidified agar proved the most useful. The ability of organism A to grow fairly rapidly at low temperatures is made use of in the method of incubating litmus milk inoculated with surface taint butter at 5.5°C. (42°F.) and then plating the milk on beef infusion agar. When an organism capable of producing surface taint was secured from a sample of butter it could be again readily secured by the same method and sometimes by other methods.

## DESCRIPTIONS OF ISOLATED ORGANISMS

The four types of organisms which were found capable of producing surface taint in butter when inoculated into pasteurized cream and the cream churned were studied morphologically, culturally and biochemically. The descriptions of these types are as follows:

### DESCRIPTION OF TYPE A

MORPHOLOGY

Form: The organisms were rod shaped.

Size: Organisms in young milk cultures varied from about 0.8 to 1.8 microns in length with a most common length being about 1.75 microns. The average width was about 0.5 micron. On young beef infusion agar slopes the organisms varied from about 0.75 to 2.25 microns with the most common length being about 1.75 microns. The average width was about 0.65 micron.

Arrangement: The organisms appeared singly and in pairs in preparations from milk and agar.

Motility: The organisms in hanging drop preparations made from young bouillon cultures were motile. Stains indicated that the flagella were peritrichous.

Staining Reaction: The organisms stained readily with the usual stains and were gram negative.

Spore Formation: Nothing resembling spores was seen in microscopic preparations. In trials in which the organisms were heated in milk, they were destroyed by 50°C. (112°F.) for five minutes.

CULTURAL CHARACTERISTICS

Agar Slope: Beef infusion agar slope cultures showed an echinulate to beaded, dull brown, viscous growth. At room temperature growth was quite evident after 24 hours, while at 37.5°C. (99.5°F.) growth was not present. Cultures grew well at 5.5°C. (42°F.) but not as rapidly as at room temperature. Old cultures at room temperature were a dark brown color.

Agar Stab: Agar stab cultures showed a heavy brown viscous surface growth, with echinate growth along the line of inoculation. Growth was abundant at room temperature. Growth was evident but much slower at 5.5°C. (42°F.). The older colonies were darker than the younger colonies and were commonly thicker at the centre.

Agar Plate Colony: Surface colonies were dull brown, viscous and myceloid with a slightly umbonate surface. Growth was evident at room temperature in a couple of days and the mature colonies were 3-5 mm. in diameter. The subsurface colonies were more or less oval and smaller than the surface colonies.

Gelatine Stab: Growth on gelatine was evident in 48 hours at room temperature. After four days liquefac-

tion was observed and was infundibuliform with a reddish-white precipitate.

Bouillons: In those bouillons in which growth occurred it was evident as a turbidity and reddish sediment. A greyish, flaky pellicle with a tendency to cling to the wall of the tube was observed. Upon shaking the tube the pellicle would settle to the bottom. Growth occurred in maltose and sucrose, but not in plant bouillon or bouillons containing galactose, glucose, mannitol, lactose, salicin, inulin or levulose.

Potato: At room temperature the growth was luxuriant, being dirty brown and raised in character. In older cultures the growth covered much of the surface and was moist, viscous and of a dark brown color.

Dunham's Solution: Growth was evident at room temperature and was observed as a cloudy precipitate with a greyish pellicle formation free from the wall of the tube. A slight reddish darkening at the surface of the solution was noted.

Uschinsky's Solution: Growth was evident at room temperature. A cloudy precipitate was observed.

Litmus Milk: The first change in litmus milk was a rapid reduction which was evident in about eight hours at room temperature. The reduction began at the bottom

of the tube. After complete reduction, proteolysis beginning at the surface of the milk was evident in 24 hours. Proteolysis continued until all the milk had been digested. Curd was not formed at any stage.

Plain Milk: Aside from the litmus reduction essentially the same changes occurred in plain milk as in litmus milk.

BIOCHEMICAL CHARACTERISTICS

Gas Production: No gas formation was observed in milk or bouillions.

Indol Production: No indol production was detected.

Acetyl-Methyl-Carbinol: No acetyl-methyl-carbinol was detected.

Reduction of Nitrates to Nitrites: The test showed a strongly positive reduction of nitrates to nitrites.

Reaction Change: In milk and those bouillions in which growth occurred the reaction was from neutral to slightly alkaline.

## DESCRIPTION OF TYPE B

MORPHOLOGY

Form: The organisms were rod shaped.

Size: Organisms in young milk cultures varied from 2 to 3.7 microns in length with the most common length being about 3.15 microns. The average width was about

0.8 micron. On young beef infusion agar slopes the organisms varied from 1.7 to 3.8 microns with the most common length being about 3.12 microns. The average width was about 0.75 micron.

Arrangement: The organisms appeared singly and in pairs in preparations from milk and agar. Occasionally thread-like chains were observed in preparations from agar.

Motility: The organisms in hanging drop preparations made from young bouillon cultures were motile. Stains indicated that the flagella were monotrichous.

Staining reaction: The organism stained readily with the usual stains. It was gram negative.

Spore formation: Nothing resembling spores was seen in microscopic preparations. In trials in which the organisms were heated in milk, they were destroyed by 62.8°C. (145°F.) for five minutes.

CULTURAL CHARACTERISTICS

Agar Slope: Beef infusion agar slope cultures showed a filiform to echinulate, yellowish-brown, glistening growth. At room temperature growth was quite evident after 24 hours.

Agar Stab: Agar stab cultures showed a heavy yellowish-brown surface growth, with filiform growth along the line of inoculation. Growth was abundant at room temperature.

Agar Plate Colony: Surface colonies were a dirty yellowish-brown and entire with a convex to slightly pulvinate surface. Growth was evident at room temperature in a couple of days and the mature colonies were 1-3 mm. in diameter. The subsurface colonies were more or less oval and smaller than the surface colonies, many were just visible macroscopically.

Gelatine Stab: Growth in gelatine was evident in 48 hours at room temperature. After seven days the liquefaction observed was stratiform with a slightly white precipitate.

Bouillons: Growth in bouillons was evident as a turbidity and slightly dirty white precipitate. A greyish, flaky pellicle with a tendency to cling to the wall of the tube was observed. Upon shaking the tube the pellicle would slowly settle to the bottom. Growth occurred in plain bouillon and bouillons containing raffinose, galactose, glucose, maltose, sucrose, mannitol, lactose, salicin, inulin, levulose and glycerol.

Potato: At room temperature the growth was fairly abundant, being a light brown color and moist. With age the potato was discolored a dirty brown, not unlike that of the colony.

Dunham's Solution: No growth was observed at room temperature.

Uschinsky's Solution: No growth was observed at room temperature.

Litmus Milk: The first change noted in litmus milk was alkalinity beginning at the surface of the milk. This change was evident in about two days time at room temperature and was followed by a digestion which was of a watery nature. The digestion gradually deepened until it reached the bottom of the tube. In about one week the digested material was of a purplish color which gradually changed to a reddish-brown color with age. Curd was not formed at any stage. The digested culture had a very objectionable odor. Growth was evident at 5.5°C. (42°F.) after one week. There was slight growth at 37.5°C. (99.5°F.).

Plain Milk: Aside from the color change noted in the litmus milk the same changes occurred in plain milk as in litmus milk.

BIOCHEMICAL CHARACTERISTICS

Gas Production: No gas formation was observed in milk or bouillons.

Indol Production: No indol production was detected.

Acetyl-Methyl-Carbinol: No acetyl-methyl-carbinol was detected.

Reduction of Nitrates to Nitrites: The tests showed a strongly positive reduction of nitrates to nitrites.

excepting two cultures which showed only a slight reduction.

Reaction Change: In milk and bouillons the reaction was alkaline.

## DESCRIPTION OF TYPE C

### MORPHOLOGY

Form: The organism was rod shaped.

Size: Organisms in young milk cultures varied from 1.3 to 2.8 microns in length with the average length being about 2.5 microns. The average width was about 0.5 micron. On young beef infusion agar slopes the organisms varied from 1.3 to 3.5 microns in length with the average length being about 2.5 microns. The average width was about 0.55 micron.

Arrangement: The organisms appeared singly and in pairs in preparations from milk and agar.

Motility: The organisms in hanging drop preparations made from young bouillon cultures were motile. Stains indicated that the flagella were monotrichous.

Staining Reaction: The organism stained readily with the usual stains. It was gram positive with some gram negative.

Spore Formation: Nothing resembling spores was seen in microscopic preparations. In trials in which the

organisms were heated in milk, they were destroyed by 62.8°C. (145°F.) for three minutes.

CULTURAL CHARACTERISTICS

Agar Slope: Beef infusion agar slope cultures showed a filiform, greyish-white growth. At room temperature growth was evident after 24 hours.

Agar Stab: Agar stabs showed a greyish-yellow growth, filiform to beaded growth along the line of inoculation. Growth was abundant at room temperature.

Agar Plate Colony: Surface colonies were a dirty greyish-yellow glistening color. They were slightly raised and somewhat ameboid. Growth was evident in about two days at room temperature and the mature colonies were from very small to 3 mm. in diameter. Subsurface colonies were very small.

Gelatine Stab: Growth in gelatine was evident in from one to two days at room temperature. After seven days the liquefaction observed was filiform.

Bouillons: Growth in bouillons was slight. It was evident as a turbidity. Growth occurred in plain bouillon and bouillons containing raffinose, galactose, glucose, maltose, sucrose, mannitol, lactose, salicin, inulin, levulose and glycerol.

Potato: At room temperature growth was scant, being a light grey color.

Dunham's Solution: No growth was observed at room temperature.

Uschinsky's Solution: No growth was observed at room temperature.

Litmus Milk: The first change observed in litmus milk was an alkalinity beginning at the surface of the milk, which was followed by ropiness. These changes were evident after 24 hours at room temperature. Growth occurred at 5.5°C. (42°F.) but not at 37.5°C. (99.5°F.). After one week at room temperature the tube of milk was a bluish-black color with the bottom being reduced to a whitish color. After several weeks at room temperature the tube of milk showed a bluish watery digestion at the surface. Curd was not formed at any stage.

Plain Milk: Essentially the same changes occurred in plain milk as in litmus milk.

BIOCHEMICAL CHARACTERISTICS

Gas Production: No gas formation was observed in milk or bouillons.

Indol Production: No indol production was detected.

Acetyl-Methyl-Carbinol: No acetyl-methyl-carbinol was detected.

Reduction of Nitrates to Nitrites: The test showed no reduction of nitrates to nitrites.

Reaction Change: In milk and bouillons the reaction was alkaline.

## DESCRIPTION OF TYPE D

### MORPHOLOGY

Form: The organism was rod shaped.

Size: Organisms in young milk cultures varied from 1.5 to 3.3 microns in length with the average length being 3 microns. The average width was about 0.77 micron. On young beef infusion agar slopes the organisms varied from 1.8 to 3.5 microns with the average length being about 3 microns. The average width was about 0.75 micron.

Arrangement: The organisms appeared singly and in pairs in preparations from milk and agar. Occasionally thread-like chains were observed in preparations from agar.

Motility: The organisms in hanging drop preparations made from young bouillon cultures were motile. Stains indicated that the flagella were peritrichous.

Staining Reaction: The organism stained readily with the usual stains. It was gram negative.

Spore Formation: Nothing resembling spores was seen in microscopic preparations. In trials in which the organisms were heated in milk they were destroyed by 62.8°C. (145°F.) for three minutes.

CULTURAL CHARACTERISTICS

Agar Slope: Beef infusion agar slope cultures showed an echinulate to filiform, light brown growth. At room temperature growth was evident after 24 hours.

Agar Stab: Agar stab cultures showed a light brown surface growth, with filiform to beaded growth along the line of inoculation. Growth was abundant at room temperature.

Agar Plate Colony: Surface colonies were a watery greyish-brown color. They were flat to raised and slightly amebold. Growth was evident in about two days and the mature colonies were from very small to 4 mm. in diameter. Subsurface colonies were very small and just visible macroscopically.

Gelatine Stab: Growth in gelatine was evident in 48 hours at room temperature. After seven days the liquefaction observed was stratiform with a slight precipitate.

Bouillons: Growth in bouillons was evident as a turbidity and a flaky white precipitate. A heavy greyish pellicle with a tendency to cling to the wall of the tube was observed. A whitish-pink precipitate was observed at the bottom of the tube. Growth occurred in plain bouillon and bouillons containing raffinose,

galactose, glucose, maltose, sucrose, mannitol, lactose, salicin, inulin, levulose and glycerol.

Potato: At room temperature the growth was abundant, being yellowish-brown and moist.

Dunham's Solution: Growth was observed at room temperature as a turbidity.

Uschinsky's Solution: No growth was observed at room temperature.

Litmus Milk: The first change in litmus milk was reduction of the litmus which was evident in about 15 to 18 hours at room temperature. Proteolysis beginning at the surface of the milk was evident in about three days. Proteolysis continued until all the milk had been digested to a reddish-brown color with a disagreeable odor. Growth occured at 5.5°C. (42°F.) but not at 37.5°C. (99.5°F.).

Plain Milk: Aside from the litmus reduction essentially the same changes occurred in plain milk as in litmus milk.

BIOCHEMICAL CHARACTERISTICS

Gas Production: No gas formation was observed in milk or bouillons.

Indol Production: No indol production was detected.

Acetyl-Methyl-Carbinol: No acethy-methyl-carbinol was detected.

Reduction of Nitrates to Nitrites: The test showed a strongly positive reduction of nitrates to nitrites.

Reaction Change: In milk and bouillons the reaction was from neutral to slightly alkaline.

The descriptions of the organisms do not agree with any of the descriptions given in Bergey's Manual of Determinative Bacteriology. The study made indicates that type A and type D belong to the genus Achromobacter, while type B and type C may possibly belong to the genus Pseudomonas.

Because of the small numbers of cultures studied it was felt that a definite idea of the variations that occur with these different types was not obtained. Therefore, it seemed advisable to postpone the naming of these types until the descriptions are published. The organisms belong to a general type of rod forms that digest milk; undoubtedly many species belong to this general type and a satisfactory scheme for their classification has not yet been worked out.

Characters of the causative organisms that are important from the standpoint of the butter industry

The outstanding characters of the organisms from the standpoint of their importance in the butter industry is their ability to produce surface taint. When any one of the organisms was inoculated into pasteurized cream, and the cream churned, the resulting butter developed surface taint in from one to three days at 15.5°C. (60°F.) and from seven to ten days at 5.5°C. (42°F.). Each of the organisms when inoculated into sterile milk and allowed to remain at room temperature for a week or ten days produced a very disagreeable odor not

unlike that of surface taint butter. This suggests the usefulness of the type of change produced in milk in attempting to culture causative organisms from surface taint butter.

Tests were made on the two predominating types, namely type A and type B, to determine the effect of salt in retarding their growth in butter. Varying amounts of salt were added at the time of working the butter, and the butter stored at 15.5°C. (60°F.) or 5.5°C. (42°F.). The results indicated that in butter containing 1.5 per cent of salt and 13.2 per cent moisture surface taint would not develop. Comparable samples of butter, unsalted or containing 0.75 per cent of salt, stored under the same conditions developed the taint.

The acid tolerance of type A and type B was determined in low salted and unsalted butter by the addition of 10 per cent of butter culture to the inoculated cream just before churning. The resulting butter, when stored under the conditions already described, kept remarkedly well and at no time showed any indication of surface taint, while samples not containing butter culture readily developed the condition. Type A was also tested for acid tolerance by the addition of lactic acid to milk. Fifty cubic centimeter portions of sterile milk in flasks were acidified to different degrees by the addition of sterile lactic acid and inoculated with a 48 hour old culture of the organism. After five days incubation at room temperature cultures were made on beef infusion agar slopes.

The lowest acidity at which the growth of type A was prevented was found to be 0.30 per cent, calculated as lactic acid. From these results on the use of butter culture or lactic acid with type A and butter culture with type B it seems evident that these organisms are sensitive to acid and it is only in butter made from low acid cream that one would expect to find them.

The temperature relationships of the four types of organisms were studied at 37.5°C. (99.5°F.), 21.1°C. (70°F.) and 5.5°C. (42°F.) by inoculating tubes of sterile litmus milk from actively growing cultures. Changes in the appearance of the litmus milk were taken as an indication of growth. All types grew best at 21.1°C. (70°F.); many showed a change in litmus milk after being held over night. Type B was the only type that showed growth at 37.5°C. (99.5°F.). At 5.5°C. (42°F.) it was found that all types would grow fairly rapidly; in the case of type A a change was noted in the litmus milk in approximately five days. Growth of these types of organisms at this low temperature shows the danger of their presence in butter stored under such conditions.

## DISCUSSION OF RESULTS

The ability to reproduce surface taint down through a series of churnings by inoculating the defective butter into pasteurized cream, and the isolation of organisms from a number of samples of commercial surface taint butter indicate that organisms are responsible. While organisms capable of producing surface taint were not found in all samples of surface taint butter examined, it appeared that the presence of large numbers of organisms that were apparently unimportant complicated the whole problem. The ratio of the different types of organisms present in the defective butter at the time of its manufacture would naturally be altered through growth. Since many types of organisms do not bring about objectionable flavors or odors, their development could easily result in other types, e.g. those capable of causing surface taint, being very inconspicuous as far as numbers are concerned and still be the important types from the standpoint of causing defects.

The reason the procedure of inoculating the pasteurized cream to be churned proved successful in the production of surface taint, while direct inoculation into normal butter did not, can be explained on the basis of the distribution and intimate relationship of the bacteria to the constituents of the butter. The presence of the bacteria in the cream

during the process of churning affords a thorough distribution that would not be accomplished by merely working the organisms into the butter. Also, the moisture and air incorporated into the butter during the working may make conditions more ideal for bacterial development.

The extremely high total bacterial counts generally found in commercial surface taint butter show that conditions for growth must have been satisfactory. The growth conditions involve temperature, salt, acidity, restraining action of other types of organisms, etc. When the growth conditions are favorable and organisms capable of producing surface taint are present, the defect may develop rapidly. This relationship holds for the development of any bacteriological defect. While the flora of butter is an important consideration the factors influencing the growth of organisms are also of significance. Among these, temperature must be given special consideration as flavor and aroma defects appear in certain lots of butter because of the very unsatisfactory holding temperatures. The importance of the different factors affecting growth varies under different circumstances. For instance, temperature may be very important when butter is unsalted or contains a low salt content, while on the other hand if the salt content is high the temperature may be of less importance. The factors influencing growth cannot be

considered independently since under practical conditions it is their combined effect that determines whether or not growth will occur.

Salt is an important factor in controlling bacterial action in butter and the demand for unsalted or low salted butter may be expected to present additional keeping quality problems to the butter manufacturer. In milk certain organisms very definitely influence the development of other types and the same relationship would be anticipated in butter. There is some evidence that the use of butter culture tends to limit the development of various objectionable conditions in butter. It would be anticipated that the restraining action of acidity, as well as other types of organisms, would bring about unfavorable conditions for the development of the certain organisms. Surface taint apparently has occurred in highly salted butter and also in butter in which butter culture has been used, but most of it has been noted in low salted butter made without butter culture. The use of butter culture in buttermaking is not generally practiced in Canada, and the market demand is for butter of a low salt content. These practices may be factors in favor of the development of surface taint.

Butter contains considerable material that can be used as food by bacteria. The availability of these different nutrients for the bacteria present may be of greater im-

portance than their amounts. Fat and casein are the constituents of butter whose decomposition would be expected to result in objectionable conditions. Both are present in amounts sufficient to support extensive development of microorganisms and the products formed by such action are often undesirable from the standpoint of flavor and odor, at least as far as butter is concerned. The flavor and odor of surface taint butter are suggestive of protein decomposition and are presumably due to the breaking down of casein or some casein derivative. It would be expected that if certain types of organisms develop in butter the conspicuous change produced would involve the products of protein decomposition, while with others, products formed by the breaking down of the fat would be more prominent. The evidence of proteolysis in surface taint butter, as determined by the soluble and amino nitrogen, is significant from the standpoint of the nature of the defect.

## SUGGESTED METHODS FOR THE CONTROL OF SURFACE TAINT

The work that has been done with surface taint butter makes it possible to suggest certain methods of control. Trials made on the heat resistance of the causative organisms isolated from surface taint butters show that the common pasteurizing temperatures will destroy them. This means that with efficient pasteurization the danger comes after the heating.

The question of creamery sanitation is of paramount importance when any microbiological problem arises. The many pieces of equipment with which cream and butter come in contact during the process of manufacture afford numerous opportunities for contamination if they are not properly cleaned and sterilized. Churn sanitation has always been a problem to the creamery operator. It is the one piece of equipment in the creamery that is difficult to clean and sterilize because of the materials out of which it is made and the nature of its construction. It is quite possible for the churn to be sterile as far as yeasts and molds are concerned and yet be heavily contaminated with bacteria. This condition makes it difficult for the manufacturer to understand why organisms can be responsible for surface taint butter when a common index of sanitation, namely, numbers of yeasts and molds, shows the butter to be made under supposed-

ly sanitary conditions. Generally, the greatest contributor of undesirable organisms to butter made from properly pasteurized cream is the churn. Every precaution should be taken to keep the churn, as well as other equipment with which the butter comes in contact, properly cleaned and sterilized.

Various investigators have pointed out the danger of polluted water as a source of surface taint in butter. Only water of known microbiologic purity should be allowed to come in contact with butter. Also, equipment may become contaminated from polluted water and serve as a secondary source of infection.

The percentage of salt in butter is important in the control of surface taint. The whole problem of sanitation in buttermaking requires a great deal of attention, especially in the manufacture of unsalted and low salt butter since salt is known to influence the growth of many types of bacteria. During recent years there has developed a demand for mildly salted butter and this can only be successfully met by careful manufacturing procedures. Methods that were satisfactory in preventing deterioration in what is nowadays considered highly salted butter may prove very unsatisfactory in low salt butter because of the more favorable condition for bacterial development.

The value of butter culture in the control of surface taint should be recognized. When such a culture is employed,

either with or without actual ripening of the cream, the numbers of butter culture organisms in the butter made from it will greatly outnumber the numbers from other undesirable types that may be present as a result of contamination. Because of the great predominance of the butter culture organisms, their growth in butter, especially unsalted and low salt content butter, may have a restraining action on other types and, in some instances, may actually cause their destruction by bringing about unsuitable conditions through the development of acid.

Temperature is a big factor in the control of bacterial growth in butter as well as other materials and highly contaminated butter may keep well at low temperatures. However, it is to be expected that butter in transit to market, in retail channels, in the home, etc. will be subjected to severe conditions and this emphasizes the importance of preventing the entrance of undesirable types of organisms. The object should be to manufacture butter as free as possible from organisms and then hold it under careful conditions. The numbers of organisms that get into butter should be so small that if the butter is subjected to unfavorable temperatures for a while there will not be the danger of serious bacterial deterioration.

## CONCLUSIONS

1. The numbers of bacteria in commercial surface taint butter were generally high. The numbers of yeasts were usually high, while the numbers of molds were variable.

2. The numbers of micro-organisms were in general greater at the surface of the butter than in the interior of the butter.

3. Many of the bacteria developing on beef infusion agar plates poured with commercial surface taint butter were essentially the same as those from normal butter.

4. Acidity determinations of surface taint butter showed nothing abnormal.

5. Soluble and amino nitrogen values were greater on butter from the surface than on that from the interior.

6. Lactose determinations revealed no differences between the surface and the interior of surface taint butter.

7. Titration and colorimetric methods showed that distillates from surface taint butter were more alkaline than those from normal butter in one comparison, while in another comparison there was no difference.

8. Direct inoculation of surface taint butter into normal butter failed to reproduce the defect.

9. Surface taint was readily reproduced in butter by inoculating pasteurized cream with defective butter and churn-

ing it. The resulting butter developed a pronounced surface taint in one to three days when stored at 15.5°C. (60°F.) and in seven to ten days at 5.5°C. (42°F.).

10. Surface taint could be reproduced down through a series of churnings.

11. Often samples of high grade, low salt content, butter developed surface taint when held at room temperature for seven days.

12. Microscopic examinations of surface taint butter showed many more organisms than were revealed by the plate count.

13. In general, the organisms that were prominent on plates poured with beef infusion agar failed to produce surface taint.

14. Organisms present in small numbers in surface taint butter were secured that would produce the condition when inoculated into cream and the cream churned, but not when inoculated into normal butter.

15. Seventeen cultures of organisms, capable of producing surface taint in butter when inoculated into pasteurized cream and the cream churned, were isolated from seventeen samples of defective butter. The experimental butter developed surface taint in one to three days when stored at 15.5°C. (60°F.) and in seven to ten days at 5.5°C. (42°F.).

Six of the samples yielding an organism that caused surface taint were from Canada and eleven from the United States.

16. The seventeen cultures of organisms were of four types.

17. From other samples of surface taint butter the causative organisms could not be secured.

18. Either a high salt content or the use of butter culture prevented the development of surface taint in butter when either defective butter or one of the causative organisms were used as the inoculating material.

19. The development of surface taint in butter hinges on two factors, (1) the presence of an organism capable of bringing about certain changes, and (2) growth conditions satisfactory for its development.

20. Surface taint in butter, like so many defects in dairy products, is not always due to the same organism, but may be caused by several types that bring about essentially the same change.

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