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The relationship between flavor deterioration in butter at 210C and keeping quality at lower temperatures

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THE RELATIONSHIP BETWEEN FLAVOR DETERIORATION IN BUTTER AT 21° C. AND KEEPING QUALITY AT LOWER TEMPERATURES

By

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INTRODUCTION

Under present conditions of butter distribution considerable time is involved in the movement of butter from the manufacturing plant to the consumer. Even when current production is going directly into consumer channels, there may be from 2 weeks to 2 months required for the process of distribution. When seasonal production is greater than consumption, the time between manufacturer and consumer is considerably extended and may involve storage over a period of several months. In either case the question of keeping quality is one of paramount importance since deterioration in flavor means a lowering in ultimate market value.

The relative keeping qualities of various lots of butter are of utmost importance to the butter manufacturer. If the keeping qualities of various lots can be predicted it enables the manufacturer to dispose of the butter of poor keeping quality before serious losses in flavor score and, consequently, in market value have occurred.

Numerous attempts have been made to predict the keeping quality of butter on the basis of various laboratory tests. Plate counts of total, lipolytic, and proteolytic bacteria and of yeasts and molds have been studied in relation to keeping quality but no definite correlation has been established. Microscopic examinations of stained butter serum have indicated that valuable information on keeping quality may be gained by a study of the numbers and types of bacteria in the fresh butter. This method, however, is limited to the plants equipped with laboratories

manned by trained technicians. Tests based on the chemical conditions in butter, as indicated by the titrable acidity, the peroxidase content, and the catalase content, have been suggested but have not been widely applied.

A test which has been employed commercially and found to be useful under certain conditions is the holding test. This test involves the holding of small samples of butter at relatively high temperatures and observing the flavor deterioration over a period of 7 to 10 days. Since both chemical and bacteriological activities increase as temperatures rise within certain limits, it appears reasonable that a keeping quality test made at 21° C. should give considerable information on the changes which may be expected at lower temperatures. Before such a keeping quality test can be widely accepted, more definite information must be obtained on the comparative time required at different temperatures for the development of certain flavor defects. It is necessary to determine whether or not a defect occurring at 21° C. will also develop at lower temperatures when sufficient time has elapsed.

STATEMENT OF PROBLEM

A satisfactory test for keeping quality, consisting of holding small samples of butter at comparatively high temperatures, involves three important relationships: first, the time required for the development of off-flavors should be short enough at such temperatures to make the method practicable; second, the changes giving rise to the off-flavors should occur at progressively decreasing rates in butter held at lower temperatures so that accurate predictions can be made; and third, the off-flavors developing at the higher temperatures should also develop at lower temperatures when sufficient time has elapsed.

This investigation considered the time of appearance of specific flavor defects in butter held at different temperatures, and the numbers of total, lipolytic, and proteolytic bacteria present at certain stages in the holding period. The rate of deterioration and the flavor defects appearing at 21° C. were compared with the rate of deterioration and the flavor defects occurring at lower temperatures. Such comparisons were used in judging the reliability of the changes taking place in butter at 21° C. as criteria of keeping quality of butter at lower temperatures.

A comparison was made of the numbers of bacteria in salted and unsalted butter held at different temperatures to show the influence of salt on the growth of bacteria in these products. The numbers of lipolytic and proteolytic bacteria were studied to show the relationship existing between these types of bacteria and the development of specific

flavor defects. Information on such relationships would aid in determining the causes of flavor deterioration and might be an aid in predicting the keeping quality of butter.

LITERATURE REVIEW

The influence of storage temperature on the rate of flavor deterioration in butter has long been the subject of research. According to Hunziker (26), a rise in storage temperature accelerates all of the forces which operate to lower the flavor score of butter. He states, "Heat intensifies every type of butter deterioration. It hastens oxidation, it enhances the action of bacteria and enzymes, it accelerates chemical action, and it favors mold development."

Gray and McKay (15),in 1906, studied the effect of different storage temperatures on butter quality and found that -10° F. (-23.3° C.) was superior to any of the higher temperatures tried. When stored at this temperature the butter kept better, both in storage and after removal from storage, than butter stored at higher temperatures. The butter was stored for periods of 5 to 8 months.

Rogers, Thompson, and Keithley (42) compared the loss in score on butter stored at 0° F. (-17.8° C.), 10° F. (-12.2° C.), and 20° F. (-6.8° C.). The study included raw cream butter and pasteurized, ripened cream butter and pasteurized, unripened cream butter. Storage at 0° F. gave the best results. The advantage of 0° F. over 10° F. was enough to warrant the use of the lower temperature for butter storage.

A number of investigators have considered the general relationship of microorganisms to butter spoilage. Sayer, Rahn, and Farrand (44) concluded that bacteria might cause butter deterioration without any multi-

plication. Samples of salted butter held at -6° C. showed slowly increasing bacterial counts but no definite relation between this increase and flavor deterioration was detected.

Rogers, Berg, Potteiger, and Davis (41) investigated the factors which influence the change in flavor of storage butter. Experiments with raw, pasteurized, and pasteurized cream, reinoculated with cultures from raw cream, showed that microorganisms were responsible for butter defects such as woody, rancid, and unclean. The raw cream butter was the poorest after storage. No significant difference between the pasteurized and reinoculated cream butter was noted.

Washburn and Dahlberg (53) compared the changes in bacterial counts and in score of salted and unsalted butter held for 284 days at -15° F. (-26° C.) followed by 20 days at 58° F. (14.5° C.). Little, if any, relationship existed between the number of bacteria, the acidity, and the change in score of either the salted or the unsalted butter in storage.

Brown, Smith, and Ruehle (5) studied the types and numbers of microorganisms occurring in salted butter at the end of various periods of storage up to one year at 0° C. Their investigation failed to show any definite relationship between the numbers and types of organisms and the quality of butter after storage. The most common types found in off-flavored butter were liquefying yeasts and Oidium lactis.

Redfield (39) noted that the low grade butter on the market generally showed high microscopic counts of yeasts and molds. High bacterial counts were not considered to be significant because such a large proportion of the bacteria were found to be acid producing types from the butter culture

used.

Grimes (16) considered the action of bacteria, yeasts, and molds in butter stored at -6° F. (-21.1° C.). He found that from 95 to 99 per cent of the bacteria in ripened cream butter died off during 6 months of storage. The average decrease in count in pasteurized sweet cream butter during the same period was 20 per cent. The principal types which survived in the pasteurized sweet cream butter were slow reducing Streptococcus lactis, micrococci, alkali-formers, and inert types. He stated that, "There was no evidence that enzymes produced during growth or the disintegration products produced on death of microorganisms affected the keeping quality of the butter in cold storage."

Ruehle (43) concluded that the flavor ordinarily termed metallic may be produced by metals, bacteria, or added amino acids. He listed a variety of bacteria, yeasts, and molds which were encountered in butter after storage but failed to find any definite relationships between numbers or types and specific off-flavors.

Macy (30) reported a quantitative study of the microflora of butter during storage at temperatures varying from 0° to -23° C. A comparison of the bacterial counts before and after storage indicated that, in general, the unsalted samples increased in count while the salted (above 1 per cent) samples decreased.

Butter defects were considered by Virtanen (52) to be of two types: (a) non-enzymatic reactions resulting in defects such as oily and fishy; and (b) enzymatic reactions resulting in defects such as fermented, boiled, cheese-sour, putrified, and rank. He stated that enzymes causing

butter defects were as a rule produced by gelatine liquefying water bacteria. These gelatine liquefiers were characterized by their resistance to high temperatures, their inability to grow in such high acid media as ripened cream butter, and their susceptibility to the influence of salt.

Demeter and Maier (11) compared the score and microbiological composition of 500 samples of pasteurized sour cream butter which were stored at about 38° F. (3.3°) for a period of 10 days. Counts were made of the yeasts and molds, total bacteria on lactose china-blue agar, total bacteria on standard agar, and total and proteolytic bacteria on casein agar. No significant relationships between score and bacterial counts were noted except with the total counts on the casein agar. Bacterial counts above 2,000,000 per ml. on casein agar were considered indicative of objectionable bacterial types which accompanied poor quality in butter. No specific relationship prevailed between the microflora found in the butter and such flavors as rancid, cowy, oily, and unclean.

Grimes (17) attempted to correlate the flavor grade of 135 samples of butter with the acidity and microbiological condition. The butter had been held at temperatures varying from 0° to 15° C. for a period of 2 weeks. Counts were made of the total bacteria on nutrient lactose agar (pH 6.8),of liquefying bacteria on nutrient gelatine(pH 6.8), and of yeasts and molds on nutrient lactose agar (pH 3.5). No definite correlations were noted between the flavor score and either the microbiological content or the acidity of the butter. Wide variations in numbers of liquefying bacteria, yeasts and molds occurred in each grade of butter after the 2 weeks storage.

Nelson (34) observed the changes in numbers of bacteria in butter held for 7 days at 21° C. by means of the microscopic method developed by Hammer and Nelson (23) and also by the plate method. The studies showed that large numbers of gram negative rods were associated with poor keeping quality in butter but no correlation between plate counts and butter quality was noted.

Gilmour and Cruess-Callaghan (13) studied the rate of growth of microorganisms in Irish Free State butter. They found that bacteria developed rapidly in fresh cream butter held at 10° C., the most rapid growth occurring in the low salted samples.

In a study of the comparative keeping qualities of butter from clean churns and contaminated churns, Olson and Hammer (35) found no significant difference with salted butter. With unsalted butter, however, the samples from the clean churns showed keeping qualities distinctly superior to the butter from contaminated churns. The deterioration in the unsalted butter was much more rapid than in the salted butter and was more rapid at 7° C. than at 0° C.

Loftus-Hills, Scharp, and Bellair (29) considered the factors influencing the keeping qualities of Victorian, salted butter stored at 12° F. (-11.1° C.) for 3 months. They found that there was no relationship between bacterial counts on gelatine before storage and the change in butter score during storage. Similar negative results were obtained in comparing total bacterial counts, and counts of liquefiers, yeasts, molds, or coliform organisms with change in grade. Grimes and Hennerty (18) reported similar results when they stored sweet cream, salted butter at

15° F. (-9.5° C.) for 2 to 8 months.

Shepard (46) held salted and unsalted butter at 0° and 21° C. and compared the changes in numbers of bacteria with the changes in score. In salted butter the numbers of bacteria decreased at both 0° and 21° C. but no close correlation between the bacterial counts and the gradual loss in score was noted. In unsalted butter the bacterial counts increased and the flavor score decreased at both 0° and 21° C. In 22 of 25 lots of unsalted butter the first pronounced decreases in scores occurred concurrently with the first marked increases in bacterial content.

Guthrie, Scheib, and Stark (20) investigated the relationship of the numbers of total, fat splitting, and casein digesting bacteria in butter to the changes in scores on butter. The study included salted and unsalted butter made from cream of the following classes: raw sweet, pasteurized sweet, raw sour, and pasteurized sour. The samples were held at 5°, 10°, and 24° C. and plated at intervals up to 36 days. Their results showed that, "In the absence of other spoilage factors, a direct correlation seems to exist between the number of fat splitting and casein digesting bacteria and the keeping quality of the butter." All of the butter examined spoiled more rapidly at the higher holding temperatures.

That the presence of salt causes a decrease in numbers of bacteria in butter during holding at ordinary storage temperatures is indicated by the results of various investigators. Washburn and Dahlberg (53) noted a rapid decrease in bacterial counts in salted butter held at -15° F. (-26° C.) for 284 days. Grimes (16) held salted, pasteurized sweet cream butter at -6° F. (-21.1° C.) for 6 to 7 months and found an average de-

crease of 20 per cent in the numbers of bacteria during storage. Macy (30) studied 483 samples of salted butter held at various temperatures from 0° to 23° C. and found that 73.7 per cent of the samples decreased in counts during storage. A later report by Macy, Coulter, and Combs (31) indicated that there was a general decrease in numbers of bacteria, yeasts, and molds in salted butter stored at 35° F. (1.4° C.). The higher concentrations of salt effected greater reductions in numbers, especially in the case of yeasts and bacteria. Hammer and Hussong (22) held salted butter at 7° and 21° C. and found that the numbers of bacteria decreased at both temperatures. Shepard (46) reported that, in general, the numbers of bacteria in salted butter decreased during holding at either 0° or 21° C.

In contrast to the reports of decreases in numbers of bacteria in salted butter are the findings of Gilmour and Cruess-Callaghan (13), and Grimes and Hennerty (18). The former reported that bacteria developed rapidly in salted, fresh cream butter held at 10° C.; however, the most rapid growth occurred in the low salted samples. Grimes and Hennerty held salted, sweet cream butter at 15° F. (-9.5° C.) for periods varying from 2 to 8 months. They found that the samples generally showed increases in bacterial counts. The apparent disagreement with the findings of the majority of the investigators may be partly explained by the fact that the butter samples used in the study had relatively low salt contents (from .5 to 2.0 per cent). Thirty of the 49 churnings had less than 1.5 per cent salt.

Washburn and Dahlberg (53) concluded that the numbers of bacteria

in unsalted butter decreased more rapidly at-15 ^{o} F. (-26^{o} C.) than in salted butter and increased more rapidly at 58^{o} F. (14.5^{o} C.). Macy (30) studied the bacterial counts of 122 samples of unsalted butter held at temperatures from 0^{o} to -23^{o} C. and found that 72.1 per cent of the samples showed increases in counts on holding, while 27.9 per cent of the samples showed decreases. Hammer and Hussong(22) reported increases in bacterial counts in unsalted butter held either at 7^{o} or 21^{o} C. and Shepard (46) noted increases in unsalted butter at 0^{o} and 21^{o} C.

Microorganisms are considered to be of importance in the development of certain flavor defects. The principal defects of microbial origin are rancid, cheesy and unclean.

The exact nature of the butter flavor defects referred to in the literature as rancid is somewhat uncertain. Some of the early investigators no doubt referred to all butter which had turned bitter or strong as rancid. In this review only those investigations which deal with hydrolytic rancidity are reported. Guthrie (19) differentiated between hydrolytic rancidity and oxidative rancidity in butter and asserted that only hydrolytic rancidity in butter should be termed rancidity. This flavor defect was characterized as giving the odor of butyric acid.

A number of investigators have indicated that microorganisms are an important cause of rancidity in butter. In an early reference, Browne (6) differentiated between the rancidity of whole butter and of pure butterfat. The rancid condition in the whole butter was characterized by the development of acidity, while rancidity in pure butterfat involved a chemical change in the fat. The author concluded that microorganisms could produce rancidity in whole butter but were unable to produce the defect

in pure butterfat. Reinmann (40) found that the addition of antiseptics prevented spoilage in butter at room temperature. This fact supported the view of bacterial rather than chemical causes of spoilage. From a review of the results of various investigators on the subject of fat decomposition by microorganisms, and from his own studies, Schreiber (45) concluded that: (a) pure butterfat by itself is not a food for microorganisms; (b) a number of bacteria which occur in nature have the ability, in the presence of food oxygen, not only to split fat but to destroy it; (c) the breakdown of fat is more rapid the finer the fat is emulsified; and (d) the splitting of fat by bacteria and molds is greatly limited by anaerobic conditions.

Orla-Jensen (36) isolated a number of types of organisms which were associated with rancidity in butter. Some of the most common types found were Oidium lactis, Cladosporium butyri, Mycoderma varieties, lactose fermenting yeasts, lactobacilli and Penicillium glaucum. His work indicated that these microorganisms and oxygen of the air were the chief causes of rancidity. Sayer, Hahn and Farrand (44) failed to find any relationship between the microflora of butter and the development of rancidity. They held butter at -6° C. and studied the changes in numbers of both total and lipolytic bacteria. The lipolytic bacteria were determined by the use of litmus lactose agar containing fat. Very few of the samples of butter contained fat splitting organisms, as indicated by the method used, and no correlation was noted between the number of lipolytic bacteria and deterioration.

Investigations by Stokoe (50) into the cause of rancidity in butter

and oleomargarine indicated that this defect in both products was caused by microorganisms. Gratz (14) concluded that microorganisms in butter were of prime importance in the development of rancidity. He found that the numbers of lipolytic microorganisms and the activities of their enzymes determined the fat splitting. Oidium lactis was found to be one of the most active agents in the development of rancidity.

Collins (7) studied the changes in numbers of lipolytic bacteria in unsalted, raw cream butter at 6° C. He concluded that, although there was at first a rapid increase in bacteria as rancidity developed, there was later a progressive and rapid development of rancidity concurrent with a rapid decrease in numbers of bacteria. His conclusions suggested the possibility of enzymatic or chemical action in the later stages of the defect. In a study of the action of lipolytic bacteria in butter, Collins (8) found that unsalted, pasteurized cream butter made in a carelessly cleaned churn very frequently developed rancidity when held at 0° C. for 7 months. Large numbers of lipolytic bacteria were found associated with the defect. The actively lipolytic bacteria isolated from rancid butter were inhibited by more than 1 per cent salt in butter.

Hussong (28) isolated Pseudomonas fragi from rancid butter and found that this organism was also widely distributed in milk, cream, and other dairy products. His results indicated that the organism increased rapidly in unsalted butter and brought about a rancid condition in as short a period as 4 days at 21° C.

Olson and Hammer (35), in a study of the keeping qualities of butter from clean and contaminated churns, found that rancidity was the most

common flavor defect developing in unsalted butter from contaminated churns. The butter was held at 32° F. (0° C.) and 45° F. (7.2° C.) and scored at various intervals until definite flavor defects developed. Rancidity developed much more quickly in the samples held at 45° F. than those held at 32° F.

Hammer and Collins (21) studied the numbers of lipolytic bacteria in butter by the nile-blue sulphate method. They found comparatively few lipolytic bacteria in fresh, lightly salted butter of good quality, such as exhibition butter. In 24 lots of butter held at 0° to 10° C. the counts varied from less than 1,000 to 40,000 per ml. In the ten samples in which lipolytic bacteria were detected, from 0.3 to 18.5 per cent of the total bacteria were lipolytic. In 12 samples of unsalted pasteurized cream butter held at 0° C. for 7 months, the numbers of lipolytic bacteria varied from 6,000 to 12,000,000 per ml. and from 0.1 to 23.5 per cent of the total bacteria were lipolytic; all of these samples showed some rancidity after storage.

Further studies by Collins and Hammer (9) included a comparison of the lipolytic action of certain bacterial cultures on beef infusion agar containing fat emulsion and in unsalted butter held at 21° C. Eighty cultures which hydrolyzed fat dispersed in beef infusion agar plates, as shown by nile-blue sulphate, were studied for their action on unsalted butter at 21° C. Of these 80 cultures, 60 (75.0 per cent) produced rancidity in the butter. Certain of the cultures of lipolytic bacteria which produced rancidity in butter produced another defect along with rancidity. Out of a total of 159 lipolytic cultures studied, 102 (64.2 per

cent) very evidently proteolyzed milk, as shown by litmus milk cultures.

Shepard (46) used the nile-blue sulphate method to detect the numbers of lipolytic bacteria in butter held at 0° and 21° C. Marked deterioration of the unsalted butter occurred at 0° C.; the most common flavor defects noted were rancidity and cheesiness. No correlation between the numbers of lipolytic bacteria and the appearance of rancidity was noted.

Proteolysis induced in storage butter by microorganisms has been considered an important cause of off-flavors by a number of investigators. Rahn, Brown, and Smith (38) showed that an increase in amide nitrogen occurred in all samples of butter during storage concurrently with a decrease in flavor score. Although the numbers of microorganisms increased slowly in the butter held at -6° F. (-21.1° C.), no correlation between the numbers of microorganisms and flavor deterioration could be noted. Brown (4) found that casein decomposition occurred in both salted and unsalted butter in storage and suggested that at least a part of this decomposition was caused by the bacterial flora of the butter. Hunziker, Spitzer, Mills, and Switzer (27) reported that protein decomposition was greater in raw cream butter than in pasteurized cream butter. They concluded that proteolysis was accelerated by microorganisms and enzymes, acids, salts, and metals through catalytic action. The microorganisms and enzymes which were active in the raw cream butter were rendered inactive in the pasteurized cream butter by the pasteurization process.

The action of specific organisms in milk and synthetic butter was determined by Spitzer, Parfitt, and Epple (48). The lots of synthetic butter, inoculated with selected pure cultures and held at 0° to 4° C.

for 120 days, showed increases in protein degradation products during storage. No attempt to judge flavors in these samples was reported.

Spitzer, Parfitt, Manhart, and Epple (49) observed that the quality of butter decreased in proportion to the protein hydrolysis and that proteolytic action was accelerated by proteolytic enzymes. Salting of butter had no influence in retarding hydrolysis although the growth of microorganisms was retarded. The pasteurization of cream destroyed microorganisms and salting restrained their activity but the enzymes were not destroyed. A later study by Spitzer and Parfitt (47) showed that bacterial cultures inoculated into butter tended to increase proteolysis, as indicated by an increase in nitrogen not precipitated by phosphotungstic acid. The numbers of total and gelatine liquefying bacteria were determined on 69 samples of contest butter held for 3 months at 0° to 4° C. All the samples which decreased in score during storage also showed increases in proteolytic counts. The greatest increases in both total and proteolytic counts occurred in the butter of lowest salt content.

Hammer and Patil (24) studied the proteolytic activity of various strains of Streptococcus lactis in milk and butter. Certain cultures evidently proteolyzed milk but showed no proteolysis in butter at any of the storage temperatures employed. The authors concluded that strains of Streptococcus lactis causing proteolysis in milk were of no significance from the standpoint of the keeping quality of butter.

Among the butter defects caused by enzyme action, Virtanen (52) listed fermented, boiled, cheese-sour, putrified, and rank. He indicated that, as a rule, the enzymes causing these defects were produced by gel-

atine liquefying water bacteria, although yeasts and molds were also possible sources.

In a consideration of the various factors influencing the deterioration of storage butter, Bendixen and Ellington (2) observed that neither the counts of proteolytic bacteria nor the increases in the products of proteolysis could be correlated with the losses in score.

Indications that cheesiness is a common defect of unsalted butter are presented in the report of Derby and Hammer (12). By inoculating cream with cultures of bacteria isolated from surface taint butter they found that cheesy flavors, associated with protein decomposition, were produced. Nelson (34) noted that the most common defects encountered in butter samples held at 21° C. for 7 days were protein decomposition, cheesiness, and putrid. Microscopic examinations made before and after the holding period indicated that bacteriological rather than chemical deterioration was responsible for the defects developed. Olson and Hammer (35) observed that unsalted butter from clean churns very frequently became cheesy after storage at either 32° F. (0° C.) or 45° F. (7.2° C.).

Herried, Macy, and Combs (25), in an exhaustive study of the microbiology of cheese-like flavors in unsalted butter, found that microorganisms capable of producing cheesiness were widely distributed in raw cream. The predominating bacteria found in mixed cultures capable of producing cheesy flavors of the Cheddar type were gram negative rods. In some cases pure cultures of bacteria isolated from cheesy butter, when inoculated into cream, were able to induce cheesiness in unsalted butter.

Artificially-mixed cultures were more consistent while naturally mixed cultures were most consistent in this respect. The development of Cheddar cheese flavors in cream or unsalted butter occurred most typically at 10° C. or lower.

The importance of microorganisms in the development of butter flavors, other than rancidity and cheesiness, has not been well established. Cusick (10) produced fishy flavor in butter by inoculating the cream with Bacterium icthyosmius (Proteus icthyosmius). The decomposition products of lecithin were considered to act as pabulum for the growth of the organisms which ultimately formed trimethylamine and gave the fishy flavor. Supplee (51) considered the bacterial counts of fishy and non-fishy lots of butter but found no correlation between counts or types and fishy flavor.

Numerous tests for keeping quality of butter have been investigated, many of which have employed either the types and numbers of microorganisms or their products as criteria of keeping quality. Certain investigators have based the prediction of keeping quality on the changes appearing in small samples of butter held at relatively high temperatures. Bouska and Brown (3) suggested a keeping quality test consisting of observing the changes in small samples of butter held at 15.5° to 21.5° C. for 7 days. Their results showed that butter of good keeping quality had a satisfactory flavor after as long as 2 weeks.

Macy and Richie (32) reviewed the results of numerous investigations dealing with the relationship of yeast and mold counts to the keeping quality of butter. They also studied the yeast and mold counts of 597

lots of commercial butter. From the review of previous work and their own studies they concluded that no definite prediction of keeping quality could be made on the basis of yeast and mold counts on fresh butter. The samples of butter with low yeast and mold counts showed slightly better keeping qualities as a group than those with higher counts. The yeast and mold counts of individual samples, however, did not serve as a reliable index to keeping quality.

Minster (33) held samples of butter at 37° C. and also measured the catalase and reductase contents of the butter to give what he termed a keeping quality value. Butter samples which showed high catalase and reductase content and developed off-flavors at 37° C. were found to have poor keeping quality at lower temperatures.

Nelson (34) employed the microscopic examination of stained butter serum and a "holding test" consisting of holding small samples for 7 days at 21° C. as bases for forecasting keeping quality of butter. These tests were used on 303 lots of commercial salted, 93 lots of commercial unsalted, and 53 lots of exhibition butter. The microscopic examination was made on the fresh butter for the purpose of indicating the numbers and types of bacteria present. From the results of the microscopic examination and of the "holding test", the keeping quality was accurately predicted in 96.4 per cent of the commercial salted, 79.6 per cent of the commercial unsalted, and 84.9 per cent of the exhibition butter. When numerous clumps of rods were present in the stained butter serum, it was almost always a sign of poor keeping quality. The author concluded further that the high microscopic counts associated with the defects devel-

oped in butter indicated that the deterioration was biological rather than chemical.

GENERAL METHODS

Source of Butter

The butter used in this work was taken from regular commercial churnings. Cream which contained in excess of 0.25 per cent acid was neutralized to about 0.21 per cent. All cream was pasteurized by the holding method at 62.5°C. for 30 minutes. Churning was carried out in commercial churns of 100 to 750 pound capacity.

All of the trials in which bacteriological studies were made included both salted and unsalted butter. The samples of unsalted butter were obtained at the time of the first moisture test while the samples of salted butter represented the finished product. Salt was added at such a rate that the finished butter contained approximately 2.5 per cent.

Holding Conditions

The samples, which were subjected to the various holding conditions, consisted of 5 ounce portions of butter taken directly from the churns with sterile, wooden spatulas, and then placed in sterile screw top glass jars with sterile parchment papers between the butter and the tops. The samples were then subjected to the prescribed holding temperatures. For Section A, these were 21°, 15°, 5°, 0°, and -25° C. The 21° and 15° C. holding cabinets were thermostatically controlled to within ± 1° C. of the required temperatures. The 5°, 0°, and -25° C. storage rooms were equip-

ped with brine refrigeration coils and the temperatures in these rooms fluctuated within the usual limits found in rooms used for holding butter.

The holding temperatures employed in Section B were "room" (21° to 26° C.), 5°, and 0° C. The 5° C. holding chamber was thermostatically controlled to within ±1° C. while the 0° C. storage room was a regular holding room equipped with brine refrigeration coils.

Sampling Methods

Every precaution was taken to prevent the contamination of the samples. Sterile wooden spatulas were used for removing all samples for plating and flavor inspection. The samples were kept in the laboratory only for the few minutes required for flavor examination and sampling. This was considered of importance because of the possible detrimental influence of warming samples to room temperature at each sampling.

The butter was examined for flavor deterioration and bacteriological condition on a regular time schedule. No attempt was made to obtain bacterial counts when flavor defects were first noted, because of the difficulty in judging the first appearance of off-flavors in a sample. The butter samples were plated when fresh and after the following holding periods:

21° C. holding after 2, 4, and 7 days.

15° C. holding after 7, 14, 21, and 28 days.

5° C. holding after 7, 14, 21, 28, and 56 days.

0° C. holding after 14, 28, and 56 days.

-25° C. holding after 1 and 90 days.

Bacteriological Methods

The methods used in all plating procedures were adaptations of those described by the Committee Report on Suggested Methods for the Microbiological Analysis of Butter (1). The small portions of butter for plating included both surface and subsurface material and were obtained by the use of sterile wooden spatulas. The samples were melted by placing the petri dishes containing them on dilution bottles that had been heated to 45° to 50° C. and put in a horizontal position. This method precluded overheating and possible destruction of some of the less heat tolerant forms.

Total bacterial counts were made on beef infusion agar. For the detection of proteolytic and lipolytic bacteria a second set of plates was poured from the same dilution blanks using beef infusion agar to which was added nile-blue sulphate solution, fat emulsion, and milk. The fat emulsion was prepared by adding 5 ml. of Wesson Oil to 100 ml. of 0.5 per cent agar; this mixture was sterilized by autoclaving at 15 pounds for 20 minutes and after cooling to 20° to 25° C. it was shaken until a fine emulsion was produced. The nile-blue sulphate solution was made by dissolving 2 grams of nile-blue sulphate in 1,000 ml. of distilled water and sterilizing in the usual manner. The materials were added to the agar in the following amounts. To each 100 ml. of beef infusion agar, there was added 2 ml. of fat emulsion, 5 ml. of 0.2 per cent nile-blue sulphate solution, and 5 ml. of sterile skimmed milk.

As noted by Hammer and Collins (21), a more intense pink color in the fat is produced by holding the fat and nile-blue sulphate in the hot agar medium for a few minutes before pouring the plates. This method was used

in the present work and aided materially in the differentiation of lipolytic and non-lipolytic colonies on the plates. The fat globules which were not lipolyzed remained pink while those which were lipolyzed appeared deep blue in the medium. The skimmed milk was added at the time of pouring the plates. It was noted that a troublesome precipitate was sometimes formed when the milk was held in the presence of the nile-blue sulphate for any length of time. Adding the milk just at the time of pouring effectively prevented the formation of such a precipitate.

The plates for total counts, as well as those for lipolytic and proteolytic counts, were incubated for 3 to 4 days at 21° to 25° C. In Section A the counting was done with the aid of a wide field binocular microscope with 6x magnification, while in Section B a Buck colony counter with 2.5x magnification was used.

The colonies which produced clear areas in the medium to which milk had been added were counted as proteolytic. The colonies which effected a change in the dispersed fat from pink to blue were reported as lipolytic. When colonies of doubtful proteolytic or lipolytic reaction appeared on the plates, they were cultured by streaking on plates to permit more opportunity for inspection. Transfers were also made into litmus milk tubes and the tubes incubated for 5 to 7 days at 21° to 25° C. In most cases the lipolytic colonies were active casein digesters, as noted on the plates and also in the litmus milk cultures.

Expression of Data

Wherever the type of data would permit, the comparisons of bacterial counts were made on the basis of the geometric means. This method was selected because it interpreted the relationship between the different sets of data more accurately than the arithmetic averages. Obviously, such a method could be applied only with studies in which definite numerical data were recorded on each sample in a series. This limited the application of the method to the tables of total bacterial counts.

The geometric mean was determined by adding the logarithms of the numbers of bacteria, dividing by the number of counts, and finding the anti-log of the quotient. The anti-log was the geometric mean, or G. M., as noted in the tables.

Methods of Flavor Inspection

The butter was examined for flavor deterioration on the schedule used in making the bacteriological examinations. The samples were not scored but were described as satisfactory or defective, according to the flavors noted. The type of flavor defect, as well as the intensity, was recorded. Particular attention was given to the detection of incipient rancidity and cheesiness, since these defects are typical microbial defects. When no definite flavor deterioration was noted, the butter was examined again after the next time interval. The various observations were recorded to show the trend of flavor deterioration.

EXPERIMENTAL

SECTION A

FLAVOR DETERIORATION AND BACTERIOLOGICAL CHANGES IN BUTTER MADE WITH BUTTER CULTURE AND HELD AT VARIOUS TEMPERATURES

The trials reported in Section A were made on butter manufactured in the butter laboratory at Iowa State College over a period extending from December to June. The cream was received from farmers in the vicinity and was of good quality. Eighteen of the twenty-five churnings included in Section A were made from sweet cream while seven were made from cream ranging in acidity from 0.30 to 0.60 per cent. Butter culture was added to all churnings at the rate of 7 per cent in the pasteurized cooled cream and the mixture of cream and culture held at about 4.4° C. until churned. In fifteen of the churnings both salted and unsalted butter samples were obtained and studies were made of the bacteriological changes and flavor deterioration. From the remaining churnings, only the unsalted butter was used and a study was made of flavor deterioration only.

Part I

Comparison of Changes in Bacterial Content and of Flavor Deterioration in Butter and in Butter Serum Held at 21° C.

The more rapid growth of bacteria when the serum is separated from butter than when the butter is in a normal condition, as indicated by the results of Hammer and Hussong(22), suggests the possibility of using the serum of butter in determining the keeping quality.

The trials in Part I were carried out to compare (a) the changes in numbers of bacteria in butter and in serum during 7 days at 21° C. and (b) the type and rate of flavor deterioration in these products.

The butter, obtained directly from the churn, was divided into two portions, one of which was held in a normal condition while the other was used as a source of the serum. The serum was separated from the butter by heating a 5 ounce portion in a water bath held at 40° to 45° C. A fairly complete separation was obtained in a period of approximately 20 minutes. The serum was drawn off with a sterile pipette and transferred to a sterile, screw top, glass jar. The butter and butter serum were plated for total, lipolytic, and proteolytic bacteria and then placed at 21° C. After 2, 4, and 7 days the samples were again plated and were also examined for flavor and oder.

Changes in numbers of total bacteria and the appearance of flavor defects in butter and in the corresponding serum at 21° C.

Table I presents the total bacterial counts and the time of appearance of flavor defects with nine churnings of unsalted butter and unsalted butter serum held 7 days at 21° C. In general, the bacterial counts increased in both the unsalted butter and the serum at 21° C. but there was considerable variation among churnings in the time at which the highest counts were reached. In butter the highest counts were recorded as follows: after 2 days with four lots, after 4 days with two lots, and after 7 days with three lots. In the serum the highest counts were recorded after 2 days with three lots, after 4 days with one lot, and after 7 days with five lots. The highest count recorded on butter was 186,000,000 per ml. while the highest count recorded on serum was 4,020,000,000 per ml.

A comparison of the magnitude of the counts in the unsalted butter and in the corresponding serum indicates that the numbers of bacteria in the serum were much greater than would be expected from the ratio of approximately 5:1 existing between whole butter and the serum content of butter. This condition may be explained by the greater availability of oxygen and nutrients for bacterial growth in the serum. As indicated by Rahn and Boysen (37), a considerable number of the moisture droplets in butter must be sterile and therefore the nutrients available for bacterial growth are limited to those contained in infected moisture droplets. In the serum, however, the moisture is the continuous phase and nutrients are available to a much greater degree than in butter.

No definite correlation between the bacterial counts and the incidence of flavor defects of individual lots could be noted in either the

Table I

Changes in Numbers of Total Bacteria and the Appearance of Flavor Defects in Unsalted Butter and the Corresponding Serum Held 7 Days at 21° C.

Definite flavor defects indicated by *

Churning no.		Numbers of bacteria per ml.			
		0 days	2 days	4 days	7 days
1	Butter	780,000	8,400,000	15,800,000	5,400,000
	Serum	76,100,000	8,200,000*	72,000,000*	282,000,000*
2	Butter	540,000	26,700,000	186,000,000	38,000,000
	Serum	810,000	111,000,000	476,000,000*	830,000,000*
3	Butter	3,140,000	91,000,000	19,000,000	6,000,000*
	Serum	11,500,000	123,000,000*	165,000,000*	450,000,000*
4	Butter	420,000	44,000,000	42,000,000*	67,000,000*
	Serum	970,000	556,000,000	579,000,000*	4,020,000,000*
5	Butter	540,000	6,400,000	5,400,000	19,800,000*
	Serum	3,200,000	188,000,000*	351,000,000*	2,550,000,000*
6	Butter	104,000	28,500,000	44,000,000	69,000,000*
	Serum	530,000	940,000,000*	590,000,000*	750,000,000*
7	Butter	640,000	17,700,000	16,300,000	12,600,000*
	Serum	24,800,000	1,170,000,000	1,150,000,000*	1,060,000,000*
8	Butter	2,030,000	43,000,000	12,300,000	14,800,000*
	Serum	23,200,000	840,000,000*	810,000,000*	176,000,000*
9	Butter	2,640,000	29,900,000	21,800,000	20,000,000
	Serum	26,600,000	287,000,000	776,000,000*	197,000,000*

unsalted butter or serum. All of the serum samples developed extremely high counts and all showed flavor deterioration. All of the lots of butter increased rapidly in counts but only six of the nine lots deteriorated in flavor. The counts on the lots which deteriorated were not significantly different from the counts on the lots which kept.

Table II presents the total bacterial counts and the time of appearance of flavor defects with nine churnings of salted butter and salted butter serum held 7 days at 21° C. A comparison of the bacterial counts on salted butter and serum indicates that the trends in counts were quite different in the two products. With the butter there was a general trend toward lower counts, while with the serum there was a marked trend toward higher counts. Seven lots of butter showed decreases in counts and two lots showed small increases after 7 days holding, while eight lots of serum increased in counts and only one lot showed a decrease after 7 days holding.

The preservative action of salt in butter was very evident, while in eight of the nine lots of serum the salt apparently prevented bacterial growth for only the first 2 to 4 days of the holding period. The types developing on plates made from the salted butter serum indicated that the acid producing bacteria of the butter culture, S. lactis, became acclimated to the conditions in the salted serum and showed considerable increase in numbers at the 4 and 7 day periods. Although the salt effectively prevented flavor deterioration in butter, it failed to prevent flavor deterioration in four of the nine serum samples.

A summary of the changes occurring in the numbers of bacteria in

Table II

Changes in Numbers of Total Bacteria and the Appearance of Flavor Defects in Salted Butter and the Corresponding Serum Held 7 Days at 21° C.

Definite flavor defects indicated by *

Churning no.		Numbers of bacteria per ml.			
		0 days	2 days	4 days	7 days
1	Butter	346,000	50,000	125,000	151,000
	Serum	5,300,000	75,000	1,700,000*	7,360,000*
2	Butter	111,000	54,000	152,000	17,500
	Serum	245,000	117,000	210,000	56,300,000*
3	Butter	213,000	10,000	2,500	6,000
	Serum	1,390,000	58,000	1,440,000	145,000,000
4	Butter	64,000	31,000	32,000	250,000
	Serum	104,000	380,000	44,400,000	98,000,000
5	Butter	330,000	120,000	11,000	3,500
	Serum	360,000	111,000	250,000	43,200,000
6	Butter	18,000	8,800	96,000	199,000
	Serum	84,000	196,000	19,200,000	174,000,000
7	Butter	196,000	108,000	93,000	84,500
	Serum	1,490,000	940,000	143,000	21,900,000
8	Butter	295,000	309,000	344,000	130,000
	Serum	3,150,000	238,000	680,000	260,000*
9	Butter	390,000	263,000	146,000	107,000
	Serum	1,027,000	1,420,000	6,500,000	29,900,000*

butter and butter serum is presented in Table III. It is evident from these results that a very different series of changes occurred in the butter and in the serum separated from it. The bacterial counts on unsalted butter increased during the first 2 days but decreased slightly during the remainder of the 7 day period while the bacterial counts on the unsalted serum showed a similar marked increase at 2 days and continued to show a significant increase to the end of the 7 day period. The counts ranged much higher and the increases in counts persisted over a longer period in the case of the unsalted serum than in the case of the corresponding butter. The numbers of bacteria in salted butter decreased abruptly during the first 2 days and then decreased more slowly to the end of 7 days while the bacteria in salted butter serum decreased during the first 2 days but then increased sharply to the end of 7 days.

The above results agree, in general, with those of Hammer and Hussong (22) for the holding period which they investigated. Their results covering the changes in bacterial counts during 2 days with the fat and serum of butter separated, but still in contact, showed that the separation of serum increased the rate of growth in the unsalted lots and increased the rate of destruction in the salted lots. These results, dealing with salted serum, apparently disagree with the results of the present trials. The increases in counts on salted serum, in the trials reported in Table III, however, occurred largely after the 2 day holding period. As shown in Table II, four of the nine lots showed increases over the initial counts at 4 days while eight showed increases at the end of 7 days holding. The bacterial counts reported in Table III range

Table III

Geometric Means of Bacterial Counts on Butter and on the Corresponding Butter Serum Held 7 Days at 21° C.

Days held	Numbers of bacteria per ml. Geometric means of nine lots of			
	Unsalted		Salted	
	Butter	Butter serum	Butter	Butter serum
0	780,000	6,390,000	160,000	670,000
2	24,900,000	244,000,000	58,000	222,000
4	23,800,000	544,000,000	57,800	1,600,000
7	19,600,000	677,000,000	52,900	27,000,000

considerably higher, both on salted and unsalted butter and on serum separated from the same, than the counts reported by Hammer and Hussong. This is probably accounted for by the fact that the butter included in the present trial was made with butter culture, while that used by Hammer and Hussong was made without butter culture.

Flavor deterioration in butter and in the corresponding serum at 21° C.

A comparison of the flavor defects appearing in butter and in the corresponding serum held 7 days at 21° C. is presented in Table IV. Flavor defects developed in all the lots of unsalted serum and in six of the nine lots of unsalted butter. The flavor defects appeared sooner and were more pronounced in the butter serum than in the corresponding butter. Flavor defects developed in four of the nine lots of salted serum but were not noted in the salted butter within the 7 day period.

A comparison of the flavor deterioration in butter and serum recorded in Table IV and the changes in bacterial counts on the corresponding lots recorded in Tables I and II show some interesting relationships. The rapid flavor deterioration in the unsalted serum was accompanied by marked increases in bacterial counts, while the slower and less extensive flavor deterioration in unsalted butter was accompanied by smaller increases in bacterial counts. Four of the nine lots of salted serum deteriorated in flavor and in three of the four lots the bacterial counts increased extensively. The fact that the large increases in numbers of bacteria in some of the lots were not accompanied by flavor defects, however, suggests that the growth of bacteria was not the only cause of

Table IV

Comparison of the Development of Flavor Defects in Butter and in the Corresponding Butter Serum Held 7 Days at 21° C.

Churning no.	Unsalted						Salted			
	Butter		Serum				Butter		Serum	
	Days	Defect	Days	Defect	Days	Defect	Days	Defect	Days	Defect
1	7	---	2	sl. off*	7	yeasty	7	---	4	yeasty
2	7	---	4	fermented	7	cheesy	7	---	7	stale
3	7	sl. off*	4	fermented	7	putrid	7	---	7	---
4	7	rancid	4	cheesy	7	roquefort	7	---	7	---
5	7	rancid	2	bitter	7	cheesy	7	---	7	---
6	7	sl. off*	2	bitter	7	roquefort	7	---	7	---
7	7	sour	4	fruity	7	cheesy	7	---	7	---
8	7	sl. off*	2	fermented	7	metallic	7	---	7	bitter
9	7	---	4	fermented	7	cheesy	7	---	7	bitter

--- No definite flavor deterioration noted
* Slightly off in flavor

deterioration in the salted serum. Flavor defects were not noted in the salted butter and the bacterial counts decreased in seven of the nine lots.

In general, it appeared that flavor defects occurred concurrently with increases in bacterial counts, since the most extensive flavor deterioration occurred in the butter and serum which showed marked increases in counts after holding, and no flavor deterioration occurred in the salted butter in which the bacterial counts decreased. Although flavor deterioration appeared only in the butter which increased significantly in counts, there was no close agreement between flavor breakdown and the bacterial counts on individual lots.

Relationship of numbers of lipolytic bacteria to the development of flavor defects in unsalted butter and in the corresponding serum at 21° C.

One of the purposes of the trials was to study the numbers of lipolytic and proteolytic bacteria accompanying the development of specific flavor defects. Preliminary work, not reported here, showed that a system of plating only at times when flavor defects were noted, presented some difficulty. Flavor deterioration, being a gradual process, could not be definitely recorded. The system of plating at fixed intervals may have missed certain significant high and low counts but it was considered superior to an indefinite plating schedule.

Table V presents a comparison of the numbers of lipolytic bacteria in unsalted butter and butter serum with the flavor defects noted during 7 days at 21° C. The numbers of lipolytic bacteria in the butter ranged from 25,000 to 12,000,000 per ml. while the numbers in the serum ranged

Table V

Numbers of Lipolytic Bacteria Accompanying Flavor Defects in Unsalted Butter and in the Corresponding Butter Serum Held 7 Days at 21° C.

Churning no.		0 days	2 days		4 days		7 days	
		Lipolytic bacteria per ml.	Defect	Lipolytic bacteria per ml.	Defect	Lipolytic bacteria per ml.	Defect	Lipolytic bacteria per ml.
1	Butter	< 100		< 1,000		3,900		14,000
	Serum	< 100	sl. off*	< 1,000	yeasty	< 1,000	yeasty	< 10,000
2	Butter	3,500		< 1,000		< 1,000		< 10,000
	Serum	10,000		< 1,000	fermented	< 1,000	cheesy	< 10,000
3	Butter	< 100		250,000		800,000	sl. off*	25,000
	Serum	300	sour	1,900,000	fermented	750,000	putrid	650,000
4	Butter	1,000		1,480,000	sl. rancid	1,600,000	rancid	4,950,000
	Serum	3,000		7,600,000	cheesy	10,000,000	roquefort	23,000,000
5	Butter	2,000		55,000		145,000	rancid	410,000
	Serum	1,500	bitter	850,000	metallic	2,200,000	cheesy	4,400,000
6	Butter	250		6,100,000		7,400,000	sl. off	12,000,000
	Serum	1,100	bitter	3,700,000	bitter	7,000,000	roquefort	16,000,000
7	Butter	200		650,000		1,500,000	sour	165,000
	Serum	5,000		400,000	fruity	350,000	cheesy	2,000,000
8	Butter	< 100		< 1,000		< 1,000	sl. off	370,000
	Serum	150	fermented	< 1,000	metallic	< 1,000	metallic	< 10,000
9	Butter	3,600		1,600,000		1,300,000		2,800,000
	Serum	1,500	sour	xxx	fermented	850,000	cheesy	400,000

xxx Too many to count * Slightly off in flavor

from less than 10,000 to 23,000,000 per ml.

No very definite relationship between the numbers of lipolytic bacteria and flavor defects was evident in either the unsalted butter or the serum. Rancid flavors were noted in only two lots of butter and the numbers of lipolytic bacteria in these lots were not significantly higher than the numbers in some lots that were not rancid. A similar condition was noted with respect to the relationship of flavor defects and lipolytic bacteria in the serum.

Relationship of numbers of proteolytic bacteria to the development of flavor defects in unsalted butter and in the corresponding serum at 21°C.

Some of the flavor defects which developed in the unsalted butter and serum appeared to be the result of proteolysis rather than fat splitting. Table VI gives the numbers of proteolytic bacteria and the flavor defects in the unsalted butter and butter serum held 7 days at 21° C. The numbers of proteolytic bacteria were usually much greater in unsalted serum than in the corresponding butter. The unsalted butter contained from less than 10,000 to 3,500,000 proteolytic bacteria per ml. while the serum contained from less than 10,000 to 43,000,000 proteolytic bacteria per ml. Six of the nine lots of unsalted butter deteriorated within 7 days while all of the lots of serum deteriorated within 4 days.

There was some indication that the large numbers of proteolytic bacteria in unsalted serum were a factor in the flavor deterioration, since the flavor defects in serum suggested proteolytic decomposition. The relationship of proteolytic bacteria to flavor deterioration in unsalted butter, however, was not so apparent. Although large numbers of proteo-

Table VI

Numbers of Proteolytic Bacteria Accompanying Flavor Defects in Unsalted Butter and in the Corresponding Butter Serum Held 7 Days at 21° C.

Churning no.		0 days	2 days		4 days		7 days	
		Proteolytic bacteria per ml.	Defect	Proteolytic bacteria per ml.	Defect	Proteolytic bacteria per ml.	Defect	Proteolytic bacteria per ml.
1	Butter	100		< 1,000		< 10,000		< 10,000
	Serum	100	sl. off	40,000	yeasty	20,000	yeasty	xxx
2	Butter	2,500		200,000		700,000		3,500,000
	Serum	5,500		140,000	fermented	350,000	cheesy	2,100,000
3	Butter	< 100		250,000		1,900,000	sl. off*	20,000
	Serum	150	sour	3,400,000	fermented	xxx	putrid	660,000
4	Butter	1,700		1,680,000	sl. rancid	2,100,000	rancid	2,700,000
	Serum	7,000		9,200,000	cheesy	13,000,000	roquefort	17,000,000
5	Butter	1,500		5,000		90,000	rancid	550,000
	Serum	1,100	bitter	1,450,000	metallic	2,000,000	cheesy	43,000,000
6	Butter	250		< 1,000		< 10,000	sl. off	< 10,000
	Serum	< 100	bitter	5,500,000	bitter	12,500,000	roquefort	9,000,000
7	Butter	200		2,500,000		2,750,000	sour	50,000
	Serum	6,000		2,050,000	fruity	1,300,000	cheesy	3,000,000
8	Butter	< 100		<1,000		<10,000	sl. off	400,000
	Serum	100	fermented	<1,000	metallic	<10,000	metallic	< 10,000
9	Butter	3,800		400,000		700,000		3,300,000
	Serum	800	sour	xxx	fermented	1,100,000	cheesy	19,500,000

xxx Too many to count * Slightly off in flavor

lytic bacteria were found in certain lots of unsalted butter, only two of the nine lots of butter showed distinct flavor defects and these two were rancid. The apparent resistance to proteolytic action might be attributed to the predominance of butter culture bacteria in this butter. The holding temperature of 21° C. was favorable to these types and, no doubt, permitted them to multiply rapidly and preserve acid conditions which definitely inhibited proteolysis.

Lipolytic and proteolytic bacteria in salted butter and the corresponding butter serum at 21° C.

The counts of lipolytic and proteolytic bacteria in salted butter and serum are not presented. The plating of these materials, using dilutions as low as 1:10, failed to show either lipolytic or proteolytic bacteria. The presence of 2.5 per cent salt in this butter apparently prevented the development of these types and also prevented the development of the off-flavors usually attributed to them.

Part II

The Influence of Freezing Butter at -25° C. as Indicated by (a) the Numbers of Bacteria Surviving and (b) the Subsequent Changes Occurring in the Butter at 21° C.

Trials were carried out to study the influence of freezing on the numbers of bacteria in salted and unsalted butter. The samples of butter, in 5 ounce glass jars, were placed at -25° C. soon after churning, except for lot No. 1 which was held at 0° to -4° C. for the first day and then placed at -25° C. Plate counts were made on the fresh butter and on the butter after 1 day and 90 days at -25° C. To avoid the effect of melting and refreezing, the jars were removed from the sharp room at the first examination for only the few minutes required to obtain samples for analyses. Each portion plated included surface and subsurface butter obtained by means of a sterile metal spatula.

After being frozen for 90 days the butter was held for an additional 7 days at 21° C. to compare the changes in numbers of bacteria and the development of flavor defects in the frozen butter with the results obtained on portions of the same butter placed at 21° C. when fresh. Bacterial counts and flavor examinations were made after 2, 4, and 7 days at 21° C.

Changes in numbers of bacteria in butter held at -25° C. for 90 days

Table VII presents the total bacterial counts on the butter before and after freezing. The freezing effected a significant reduction in the

Table VII

Influence of Freezing at -25° C. on Numbers of Total Bacteria in Butter

Churning no.	Numbers of bacteria per ml.					
	Unsalted butter			Salted butter		
	Unfrozen	Frozen		Unfrozen	Frozen	
	0 days	1 day	90 days	0 days	1 day	90 days
1	780,000	956,000*	490,000	346,000	291,000*	15,800
2	540,000	59,000	40,000	111,000	60,000	45,000
3	3,140,000	55,000	2,100	213,000	55,000	2,100
4	420,000	70,000	52,000	64,000	41,000	30,400
5	540,000	186,000	32,000	330,000	211,000	32,000
6	104,000	14,500	15,100	18,000	9,900	12,500
7	640,000	143,000	97,000	196,000	181,000	83,000
8	2,030,000	522,000	193,000	295,000	278,000	73,000
9	2,640,000	466,000	130,000	390,000	324,000	67,000
10	360,000	105,000	9,000	55,000	27,000	10,000
11	1,180,000	380,000	166,000	280,000	178,000	93,000
12	1,530,000	194,000	129,000	272,000	173,000	160,000
13	1,620,000	160,000	210,000	130,000	69,000	6,500
14	1,330,000	327,000	169,000	175,000	167,000	69,000
15	930,000	257,000	90,000	181,000	97,000	22,000
Avg.	1,185,600	259,630	121,610	203,730	144,130	48,090
G. M.	877,000	167,000	65,000	161,000	101,000	29,400

* Held at 0° to -4° C. for 1 day

numbers of bacteria in the unsalted butter, the greatest decrease occurring during the first day at -25° C. Lot No. 1, which failed to show a decrease in count at the 1 day sampling, was the lot held at 0° to -4° C. during the first day.

Freezing caused a significant reduction in the bacterial counts on all lots of salted butter after 90 days but the reduction was less marked than with the unsalted butter. When fresh, the salted butter contained much smaller numbers of bacteria than were found in the unsalted butter due, undoubtedly, to the destructive action of salt. The comparatively small decrease in the numbers of bacteria in the salted butter after 90 days at -25° C. indicated that the salt effected some protective influence and prevented the destruction of as large a percentage of the bacteria as were destroyed in the unsalted butter. The freezing point lowering due to the salt brine, in the butter serum, appears as the best explanation of the protective action to the bacterial flora in the salted butter. The presence of salt may have prevented crushing to some extent by interfering with the complete crystallization of the moisture in the butter. The salt brine concentration was approximately 16 per cent, and a brine of this concentration has a freezing point of -12.2° C.

Lipolytic and proteolytic bacteria were not detected on the plates poured with the frozen butter, even though dilutions as low as 1:100 were regularly made. These results indicate that the small numbers of lipolytic and proteolytic bacteria present in the freshly made butter, as shown in Tables V and VI, were destroyed by the freezing process.

Changes in numbers of total bacteria in fresh butter and in frozen butter held 7 days at 21° C.

Table VIII presents the numbers of total bacteria and the time of appearance of flavor defects in the frozen unsalted butter held 7 days at 21° C. The numbers of bacteria increased rapidly; the rate and extent of increase, however, varied widely among the different churnings. The highest average number for the series was reached after 7 days. Marked flavor deterioration was noted after 7 days, but no correlation between the numbers of total bacteria and the appearance of flavor defects in individual lots could be noted.

The results obtained on the frozen, salted butter held 7 days at 21° C. are presented in Table IX. No regular increase or decrease in numbers of bacteria was noted in the frozen, salted butter held at 21° C. Ten lots showed lower counts after 7 days while five lots showed higher counts than were found at the initial plating. In general, the changes in numbers were irregular and were not of great magnitude. The low initial counts in salted butter, as compared with unsalted, indicate that the influence of salt in reducing counts was probably a more important factor than the low temperature of holding.

Table X presents a summary of the changes in numbers of bacteria in butter held at 21° C. after freezing and in the corresponding butter held at 21° C. when fresh. The numbers of bacteria increased more rapidly in the frozen, unsalted butter held at 21° C. than in the corresponding fresh, unsalted butter held at 21° C. The bacterial counts on the two types of butter were approximately the same after the 7 days, even though the ini-

Table VIII

Changes in Numbers of Total Bacteria in Unsalted Butter Held 7 days at 21° C. Subsequent to Freezing

Flavor deterioration indicated by *

Churning no.	Numbers of bacteria per ml.			
	0 days	2 days	4 days	7 days
1	490,000	7,900,000	10,400,000	6,700,000
2	40,000	87,700,000	130,400,000*	85,400,000*
3	2,100	7,700,000	10,200,000*	5,800,000*
4	52,000	17,900,000	31,900,000*	34,200,000*
5	32,000	1,690,000	4,800,000*	630,000*
6	15,100	16,000,000	27,600,000*	39,400,000*
7	97,000	16,400,000	27,000,000	29,500,000*
8	193,000	26,500,000	27,300,000	32,200,000
9	130,000	57,600,000	45,600,000	78,300,000
10	9,000	33,100,000	7,500,000	6,950,000*
11	166,000	51,900,000	133,000,000	155,000,000*
12	129,000	6,000,000	37,600,000*	79,500,000*
13	210,000	920,000	6,000,000	19,800,000*
14	169,000	8,200,000	13,700,000*	30,600,000*
15	90,000	10,200,000	11,400,000	24,100,000*
Avg.	121,600	23,314,000	34,960,000	41,872,000
G. M.	65,000	13,000,000	21,000,000	20,300,000

Table IX

Changes in Numbers of Total Bacteria in Salted Butter Held 7 Days at 21° C. Subsequent to Freezing

Churning no.	Numbers of bacteria per ml.			
	0 days	2 days	4 days	7 days
1	15,800	11,700	42,300	49,000
2	45,000	15,600	47,000	17,600
3	5,400	1,000	2,100	750
4	30,400	6,700	6,700	2,200
5	32,000	5,000	8,700	6,300
6	12,500	5,200	14,400	128,000
7	83,000	37,000	9,300	20,000
8	73,000	145,000	210,000	75,000
9	67,000	91,000	7,500	57,000
10	10,000	315,000	9,000	9,000
11	93,000	5,300	12,500	9,200
12	160,000	11,000	12,500	31,000
13	6,500	14,500	6,000	30,000
14	69,000	109,000	104,000	44,000
15	22,000	26,000	8,800	48,000
Avg.	48,300	53,300	33,400	42,380
G. M.	29,400	18,700	14,900	22,900

Table X

Changes in Total Bacterial Counts on Fresh Butter and on Frozen Butter Held 7 Days at 21° C.

Butter	Number of Churnings	Bacteria per ml. (geometric means)			
		Unsalted butter		Salted butter	
		0 days	7 days	0 days	7 days
Fresh	15	877,000	22,500,000	161,000	50,900
Frozen	15	65,000	20,300,000	29,400	22,900

tial counts on the frozen butter were comparatively low. The numbers of bacteria decreased slightly in both the fresh and frozen, salted butter and were somewhat lower on the frozen butter than on the fresh butter after 7 days at 21° C.

Flavor deterioration in fresh and frozen, unsalted butter held 7 days at 21° C.

A comparison of the flavor deterioration in fresh and frozen, unsalted butter held at 21° C. is presented in Table XI. The fifteen lots were examined after 2, 4, and 7 days. Since no off-flavors were noted previous to the 4 day examination, the table gives the data for only the 4 and 7 day examinations. A flavor defect developed in only one lot of fresh butter during the first 4 days, while six lots of frozen butter showed some flavor deterioration at 4 days. After 7 days there was no significant difference in the number of lots of fresh and of frozen butter showing flavor defects, but the flavor defects were more pronounced in the frozen butter than they were in the fresh butter. Flavor defects were detected after 7 days in certain lots of fresh butter when they were not detected in the corresponding frozen lots, and vice versa. Defects of mild and indefinite character, however, may have been overlooked. The most common defects noted were rancid and roquefort flavors; there were two lots of fresh butter and five lots of frozen butter developing one or the other of these defects.

Flavor defects were not developed in either the fresh or frozen, salted butter during the 7 days at 21° C.

Table XI

Flavor Deterioration in Fresh and in Frozen Unsalted Butter Held 7 Days at 21° C.

Churning no.	Flavor comments			
	4 days		7 days	
	Fresh butter	Frozen butter	Fresh butter	Frozen butter
1	---	---	---	---
2	---	slightly off	---	roquefort
3	---	slightly off	slightly off	slightly off
4	slightly rancid	oily	rancid	slightly bitter
5	---	---	rancid	---
6	---	slightly off	slightly off	slightly off
7	---	---	sour	---
8	---	---	slightly off	---
9	---	---	---	---
10	---	---	---	roquefort
11	---	---	slightly off	roquefort
12	---	slightly oily	slightly rancid	roquefort
13	---	---	---	roquefort
14	---	slightly oily	slightly oily	moldy
15	---	---	---	slightly oily

--- Flavor satisfactory

Lipolytic and proteolytic bacteria in frozen, unsalted butter held 7 days at 21° C.

Table XII presents the numbers of lipolytic and proteolytic bacteria in frozen, unsalted butter held at 21° C. Although lipolytic and proteolytic bacteria were not detected in the initial platings they increased rapidly in the frozen, unsalted butter when it was held at 21° C. In the ten lots which showed flavor deterioration at 7 days, the numbers of lipolytic bacteria ranged from less than 1,000 to 8,000,000 per ml. The five lots which kept contained from less than 10,000 to 1,550,000 lipolytic bacteria per ml. The numbers of proteolytic bacteria in the butter which developed flavor defects ranged from 5,000 to 34,000,000 per ml. while the lots which kept ranged from less than 10,000 to 1,900,000 per ml. No correlation between the numbers of lipolytic or proteolytic bacteria and specific flavor defects could be noted. The lots which developed roquefort flavor did not contain larger numbers of lipolytic bacteria than some of the lots which showed either indefinite flavor defects or no flavor defects. A similar lack of agreement existed between the numbers of proteolytic bacteria and flavor defects.

Neither lipolytic nor proteolytic bacteria were detected in the salted butter held 7 days at 21° C. subsequent to freezing.

Table XII

Numbers of Lipolytic and Proteolytic Bacteria and the Appearance of Flavor Defects in Frozen Unsalted Butter Held 7 Days at 21° C. (Time flavor deterioration first noted indicated by *)

Churning no.	Type of bacteria	Numbers of bacteria per ml.				Flavor defects after 7 days
		0 days	2 days	4 days	7 days	
1	Lip.	< 100	<10,000	< 10,000	< 10,000	
	Prot.	< 100	<10,000	< 10,000	< 10,000	
2	Lip.	< 100	<10,000	< 10,000*	< 10,000	roquefort
	Prot.	< 100	630,000	3,000,000	34,000,000	
3	Lip.	< 100	130,000	45,000*	10,000	slightly off
	Prot.	< 100	< 10,000	500,000	15,000	
4	Lip.	< 100	300,000	200,000*	400,000	slightly bitter
	Prot.	< 100	500,000	100,000	1,000,000	
5	Lip.	< 100	20,000	85,000	< 10,000	
	Prot.	< 100	< 10,000	< 10,000	< 10,000	
6	Lip.	< 100	xxx	4,300,000*	4,700,000	slightly off
	Prot.	< 100	xxx	100,000	< 10,000	
7	Lip.	< 100	390,000	1,400,000	500,000	
	Prot.	< 100	15,000	1,200,000	850,000	
8	Lip.	< 100	355,000	150,000	55,000	
	Prot.	< 100	100,000	135,000	25,000	
9	Lip.	< 100	5,600,000	4,900,000	1,550,000	
	Prot.	< 100	900,000	1,900,000	1,900,000	
10	Lip.	< 100	4,500	20,000	5,000*	roquefort
	Prot.	< 100	5,000	55,000	10,000	
11	Lip.	< 100	160,000	450,000	8,000,000*	roquefort
	Prot.	< 100	200,000	6,200,000	16,000,000	
12	Lip.	< 100	130,000	2,200,000*	6,000,000	roquefort
	Prot.	< 100	50,000	1,600,000	7,500,000	
13	Lip.	< 100	55,000	30,000	160,000*	roquefort
	Prot.	< 100	45,000	32,000	170,000	
14	Lip.	< 100	7,500	< 1,000*	< 1,000	moldy
	Prot.	< 100	6,500	5,000	5,000	
15	Lip.	< 100	35,000	20,000	2,100,000*	slightly oily
	Prot.	< 100	35,000	190,000	2,100,000	

xxx Too many to count with dilutions used

Part III

Changes in Numbers of Bacteria and Flavor Deterioration in Butter Held at Different Temperatures

Samples of salted and unsalted butter made with butter culture were held at different temperatures for the study of: (a) the changes in numbers of bacteria; (b) the comparative time required for flavor defects to appear; and (c) the relationship between the changes in numbers of bacteria and the appearance of flavor defects. The study of flavor deterioration and bacteriological changes involved fifteen sets of samples. Each set was taken from one churning and consisted of six or eight lots of butter, half of which were salted and half of which were unsalted. Two lots from each set, one salted and one unsalted, were then placed at each of the different holding temperatures. Platings for total, lipolytic, and proteolytic bacteria and examinations for flavor deterioration were made after definite time intervals, regardless of the progress of flavor deterioration. No data on the numbers of lipolytic or proteolytic bacteria in salted butter are presented because these types of bacteria were not noted on plates made from dilutions as low as 1:10. Flavor defects were also absent in the salted butter during the periods of observation.

Ten additional sets of samples of unsalted butter were obtained as described above and were studied only for comparative flavor deterioration at the different holding temperatures.

Changes in numbers of bacteria and in the flavor of butter held at 21° C.

Table XIII gives the total bacterial counts on the unsalted butter held 7 days at 21° C. The average counts showed marked increases up to 4 days. The highest counts on the various lots, however, were not regularly obtained at any one time but were found after 2 days with five lots, after 4 days with two lots, and after 7 days with eight lots. The ranges of counts were as follows:

Initial counts 104,000 to 3,140,000 per ml.

After 2 days 2,850,000 to 91,000,000 per ml.

After 4 days 5,400,000 to 186,000,000 per ml.

After 7 days 5,400,000 to 69,000,000 per ml.

The total bacterial counts on salted butter held 7 days at 21° C. are presented in Table XIV. The average counts on salted butter decreased during the 7 day holding period. The lowest counts were recorded after 2 days with five lots, after 4 days with two lots, and after 7 days with eight lots. With two lots, higher counts were found after 7 days than were found in the fresh butter.

The influence of salt on the numbers of bacteria in butter held at 21° C. is indicated by a comparison of the results in Tables XIII and XIV. As indicated by the G. M. before and after holding, the numbers of total bacteria in unsalted butter increased about twenty-threefold, while the numbers of bacteria in the salted butter decreased to about one-third of the initial numbers. Salt exerted a marked inhibiting action on the growth of bacteria in thirteen of the fifteen lots of salted butter.

Table XIII

Changes in Numbers of Total Bacteria in Unsalted Butter Held 7 Days at 21° C.

Churning no.	Numbers of bacteria per ml.			
	0 days	2 days	4 days	7 days
1	780,000	8,400,000	15,800,000	5,400,000
2	540,000	26,700,000	186,000,000	38,000,000
3	3,140,000	91,000,000	19,000,000	6,000,000
4	420,000	44,000,000	42,000,000	67,000,000
5	540,000	6,400,000	5,400,000	19,800,000
6	104,000	28,500,000	44,000,000	69,000,000
7	640,000	17,700,000	16,300,000	12,800,000
8	2,030,000	43,000,000	12,300,000	14,800,000
9	2,640,000	29,900,000	21,800,000	20,000,000
10	360,000	9,000,000	7,050,000	5,700,000
11	1,180,000	16,100,000	17,100,000	24,100,000
12	1,530,000	15,300,000	33,000,000	62,500,000
13	1,620,000	19,000,000	18,500,000	29,300,000
14	1,330,000	4,250,000	14,900,000	21,800,000
15	930,000	2,850,000	13,900,000	17,800,000
Avg.	1,186,000	24,140,000	31,137,000	27,600,000
G. M.	877,000	16,540,000	20,180,000	20,300,000

Table XIV

Changes in Numbers of Total Bacteria in Salted Butter Held 7 Days at 21° C.

Churning no.	Numbers of bacteria per ml.			
	0 days	2 days	4 days	7 days
1	346,000	50,000	125,000	151,000
2	111,000	54,000	152,000	17,500
3	213,000	10,000	2,500	6,000
4	64,000	31,000	32,000	250,000
5	330,000	120,000	11,000	3,500
6	18,000	8,800	96,000	199,000
7	196,000	108,000	93,000	84,500
8	295,000	309,000	344,000	130,000
9	390,000	263,000	146,000	107,000
10	55,000	8,100	9,500	13,400
11	280,000	231,000	123,000	77,000
12	272,000	105,000	127,000	115,000
13	130,000	11,400	11,500	75,000
14	175,000	182,000	124,000	175,000
15	181,000	46,800	47,000	8,000
Avg.	204,000	102,600	96,200	94,100
G. M.	161,000	88,000	53,100	51,000

The results in Table XV include the numbers of lipolytic and proteolytic bacteria and the flavor defects in unsalted butter held at 21° C. The numbers of lipolytic bacteria in the fresh butter were very low. The highest number recorded at 0 days was 3,600 per ml. and six lots contained less than 100 per ml. The numbers of lipolytic bacteria increased in fourteen of the fifteen lots but considerable variation was noted in the time at which the highest counts occurred. The numbers of lipolytic bacteria exceeded 1,000,000 per ml. in seven of the fifteen lots at some time during the 7 day holding period. Only three lots of butter became rancid and these lots did not contain significantly higher numbers of lipolytic bacteria than some of the lots which showed indefinite off-flavors or no off-flavors.

The numbers of proteolytic bacteria increased in thirteen of the fifteen lots of unsalted butter held at 21° C. The increases in two of these lots, however, were questionable because of the small numbers of colonies on which the counts were based. In those lots in which the counts increased, the highest counts were noted either at 4 days or at 7 days. Off-flavors usually associated with proteolytic activity were not detected in any of the unsalted butter even though counts exceeding 1,000,000 proteolytic bacteria per ml. were obtained on six of the fifteen lots at some time during the 7 days.

No definite correlation was noted between the numbers of either lipolytic or proteolytic bacteria and the development of flavor defects at 21° C. Neither the type nor the intensity of the flavor defect appeared to be indicated by the counts of lipolytic or proteolytic bacteria.

Table XV

Comparison of Numbers of Lipolytic and Proteolytic Bacteria and Flavor Defects in Unsalted Butter Held 7 Days at 21° C.

(Time flavor deterioration first noted indicated by *)

Churning no.	Type of bacteria	Numbers of bacteria per ml.				Flavor defects after 7 days
		0 days	2 days	4 days	7 days	
1	Lip.	< 100	< 1,000	3,900	14,000	
	Prot.	< 100	< 1,000	< 1,000	< 10,000	
2	Lip.	3,500	< 1,000	< 1,000	< 1,000	
	Prot.	2,500	200,000	700,000	350,000	
3	Lip.	< 100	250,000	800,000	25,000	sl. off
	Prot.	< 100	250,000	1,900,000	20,000*	
4	Lip.	1,000	1,480,000	1,600,000	4,950,000	rancid
	Prot.	1,700	1,680,000	2,100,000*	2,700,000	
5	Lip.	2,000	55,000	145,000	410,000	rancid
	Prot.	1,500	5,000	90,000	550,000*	
6	Lip.	250	6,100,000	7,400,000	12,000,000	sl. off
	Prot.	250	< 1,000	< 1,000	< 10,000*	
7	Lip.	200	650,000	1,500,000	165,000	sour
	Prot.	200	2,500,000	2,750,000	50,000*	
8	Lip.	< 100	< 1,000	< 1,000	370,000	sl. off
	Prot.	< 100	< 1,000	< 1,000	400,000*	
9	Lip.	3,600	1,600,000	1,300,000	2,800,000	
	Prot.	3,800	400,000	700,000	3,300,000	
10	Lip.	< 100	260,000	230,000	175,000	
	Prot.	< 100	2,000	5,000	< 10,000	
11	Lip.	< 100	155,000	600,000	1,050,000	sl. off
	Prot.	< 100	145,000	600,000	1,050,000*	
12	Lip.	450	530,000	2,500,000	360,000	sl. rancid
	Prot.	400	60,000	650,000*	700,000	
13	Lip.	< 100	4,050,000	1,500,000	1,400,000	
	Prot.	< 100	1,050,000	1,200,000	3,450,000	
14	Lip.	300	32,000	20,000	50,000	sour
	Prot.	< 100	< 1,000	< 1,000*	5,000	
15	Lip.	500	5,000	245,000	285,000	
	Prot.	< 100	5,000	75,000	550,000	

sl. = slightly

Changes in numbers of bacteria and in the flavor of butter held at 15° C.

Table XVI presents the total bacterial counts on seven lots of unsalted butter held 28 days at 15° C. In general, the bacterial counts increased during the first 14 days of the holding period. The highest counts were obtained on one lot at 7 days, on four lots at 14 days, and on two lots at 28 days. The ranges of counts were as follows:

Initial counts 360,000 to 2,640,000 per ml.

After 7 days 3,050,000 to 88,900,000 per ml.

After 14 days 17,200,000 to 157,000,000 per ml.

After 28 days 6,900,000 to 90,800,000 per ml.

The total bacterial counts on seven lots of salted butter held 28 days at 15° C. are given in Table XVII. In general, the bacterial counts decreased as indicated by the average counts after holding. The most significant change in counts occurred during the first 7 days when a decrease in numbers of bacteria was noted in six of the seven lots. The later changes in counts were irregular and indicated no marked trends in either direction.

A comparison of the results in Tables XVI and XVII shows the effect of salt on changes in bacterial counts on butter held at 15° C. As indicated by the G. M. of the counts before and after holding, the bacterial counts on unsalted butter increased about twenty-fivefold, while the counts on salted butter decreased to about two-fifths of the initial number.

Table XVIII presents the numbers of lipolytic and proteolytic bacteria and the flavor defects developed in unsalted butter held 28 days

Table XVI

Changes in Numbers of Total Bacteria in Unsalted Butter Held 28 Days at 15° C.

Churning no.	Numbers of bacteria per ml.			
	0 days	7 days	14 days	28 days
9	2,640,000	88,900,000	33,000,000	20,300,000
10	360,000	5,900,000	21,000,000	10,800,000
11	1,180,000	13,900,000	68,300,000	37,500,000
12	1,530,000	19,300,000	157,000,000	90,800,000
13	1,620,000	17,000,000	17,300,000	6,900,000
14	1,330,000	3,050,000	17,200,000	18,300,000
15	930,000	19,100,000	27,900,000	67,100,000
Avg.	1,370,000	23,900,000	48,800,000	36,030,000
G. M.	979,000	14,600,000	34,800,000	25,200,000

Table XVII

Changes in Numbers of Total Bacteria in Salted Butter Held 28 Days at 15° C.

Churning no.	Numbers of bacteria per ml.			
	0 days	7 days	14 days	28 days
9	390,000	180,000	147,000	95,000
10	55,000	8,400	8,700	4,900
11	280,000	112,000	120,000	59,000
12	272,000	126,000	130,000	183,000
13	130,000	4,500	237,000	215,000
14	175,000	176,000	190,000	210,000
15	181,000	79,000	14,000	47,000
Avg.	212,000	97,900	121,000	116,000
G. M.	183,000	54,000	59,100	72,600

Table XVIII

Comparison of Numbers of Lipolytic and Proteolytic Bacteria and Flavor Defects in Unsalted Butter Held 28 Days at 15° C.

Churning no.	Type of bacteria	0 days	7 days		14 days		28 days	
		Bacteria per ml.	Bacteria per ml.	Flavor defect	Bacteria per ml.	Flavor defect	Bacteria per ml.	Flavor defect
9	Lip. Prot.	3,600 3,800	1,900,000 1,100,000		2,950,000 1,400,000	sl.roquefort	1,300,000 3,000,000	unclean
10	Lip. Prot.	< 100 < 100	215,000 < 1,000		1,850,000 500,000	sl. rancid	150,000 15,000	roquefort
11	Lip. Prot.	< 100 < 100	70,000 45,000	sl. off	1,500,000 1,500,000	sl. rancid	3,900,000 4,500,000	sl.roquefort
12	Lip. Prot.	450 400	810,000 650,000	sl. off	1,500,000 1,450,000	roquefort	1,000,000 2,450,000	roquefort
13	Lip. Prot.	< 100 < 100	1,350,000 400,000	sl. off	600,000 500,000	sl. off	15,000 30,000	roquefort
14	Lip. Prot.	300 < 100	4,000 < 1,000		1,100,000 1,450,000	sl. rancid	2,500,000 2,500,000	roquefort
15	Lip. Prot.	500 < 100	195,000 800,000		1,000,000 1,200,000	sl. off	4,800,000 8,500,000	cheesy

sl. = slightly

at 15° C. The numbers of lipolytic bacteria increased in all lots and five of the seven lots showed off-flavors denoting lipolysis at the end of 28 days. The numbers of proteolytic bacteria increased in all lots but the maximum numbers were reached somewhat later in the holding period than was the case with the numbers of lipolytic bacteria. The highest count of proteolytic bacteria was obtained on the lot which developed a cheesy flavor at 28 days. The results indicate that lipolytic and proteolytic bacteria were relatively numerous in the lots which showed definite flavor defects.

Changes in numbers of bacteria and in the flavor of butter held 56 days at 5° C.

Table XIX presents the numbers of total bacteria in unsalted butter held 56 days at 5° C. As indicated by averages, the numbers of total bacteria in the unsalted butter at 5° C. increased to the end of 56 days. The highest counts, on individual lots, however, were obtained on two lots at 21 days, on four lots at 28 days, and on nine lots at 56 days. The ranges of counts were as follows:

Initial 104,000 to 3,140,000 per ml.

After 7 days 980,000 to 26,500,000 per ml.

After 21 days 13,200,000 to 88,500,000 per ml.

After 28 days 3,400,000 to 103,000,000 per ml.

After 56 days 3,900,000 to 90,800,000 per ml.

The numbers of bacteria in salted butter held 56 days at 5° C. are presented in Table XX. A small but definite decrease in average numbers of bacteria occurred in the salted butter at 5° C. Twelve of the fifteen

Table XIX

Changes in Numbers of Total Bacteria in Unsalted Butter Held 56 Days at 5° C.

Churning no.	Numbers of bacteria per ml.				
	0 days	7 days	21 days	28 days	56 days
1	780,000	1,030,000	19,900,000	16,200,000	90,800,000
2	540,000	11,400,000	13,400,000	19,000,000	52,000,000
3	3,140,000	980,000	83,000,000	57,000,000	47,500,000
4	420,000	10,900,000	88,500,000	37,700,000	24,000,000
5	540,000	1,320,000	25,900,000	27,000,000	34,600,000
6	104,900	6,100,000	54,000,000	79.000,000	22,600,000
7	640,000	26,500,000	21,200,000	31,000,000	64,900,000
8	2,030,000	3,900,000	18,200,000	22,800,000	33,400,000
9	2,640,000	20,300,000	43,400,000	85,000,000	66,500,000
10	360,000	1,780,000	15,400,000	22,900,000	3,900,000
11	1,180,000	16,700,000	19,200,000	13,000,000	70,900,000
12	1,530,000	17,500,000	17,500,000	24,500,000	54,200,000
13	1,620,000	4,900,000	41,100,000	26,200,000	79,400,000
14	1,330,000	2,110.000	13,200,000	3,400,000	41,400,000
15	930,000	14,300,000	79,600,000	103,000,000	79,400,000
Avg.	1,185,000	9,315,000	36,900,000	31,667,000	51,000,000
G. M.	877,000	5,590,000	29,300,000	28,200,000	42,200,000

Table XX

Changes in Numbers of Total Bacteria in Salted Butter Held 56 Days at 5° C.

Churning no.	Numbers of bacteria per ml.				
	0 days	7 days	21 days	28 days	56 days
1	346,000	182,000	12,000	8,000	12,600
2	111,000	46,000	67,000	22,000	75,000
3	213,000	136,000	3,000	950	36,000
4	64,000	69,000	37,000	30,000	270,000
5	330,000	215,000	48,000	5,000	8,000
6	18,000	19,500	18,400	60,000	82,000
7	196,000	18,400	91,000	36,000	15,000
8	295,000	304,000	215,000	185,000	15,400
9	390,000	330,000	157,000	142,000	1,050
10	55,000	42,000	12,100	6,800	11,000
11	280,000	149,000	116,000	117,000	41,000
12	272,000	178,000	155,000	170,000	130,000
13	130,000	4,900	32,000	52,000	195,000
14	175,000	263,000	154,000	179,000	71,000
15	181,000	191,000	38,000	218,000	1,600
Avg.	204,000	143,000	77,000	82,100	64,300
G. M.	161,000	88,800	46,400	37,400	26,200

lots showed lower counts after 56 days than were found on the fresh butter. It is of interest to note that lots No. 4 and 6, which increased in counts during the 56 days holding, were the same two lots which showed increases at 21° C., as recorded in Table XIV.

The influence of salt on the numbers of bacteria in butter at 5° C. is indicated by a comparison of the results in Tables XIX and XX. As indicated by the G. M. of the counts before and after holding, the numbers of bacteria increased about fiftyfold in unsalted butter while the numbers in salted butter decreased to about one-sixth of the initial numbers.

Table XXI presents the numbers of lipolytic and proteolytic bacteria and the flavor defects in unsalted butter held 56 days at 5° C. The numbers of lipolytic bacteria increased in all the lots. In fourteen of the fifteen lots the highest counts were reached after from 21 to 56 days. In general, the lots with high lipolytic counts developed typical rancidity before the 28 day examination. Lots which developed a rancid flavor contained from 150,000 to 11,000,000 lipolytic bacteria per ml. at the time the defect was first detected. Many of the lots of unsalted butter which were described as rancid had a "May apple" aroma. The "May apple" aroma on the butter and also on plates made from the butter suggested that the lipolysis was frequently caused by Pseudomonas fragi.

The numbers of proteolytic bacteria increased in fourteen of the fifteen lots of butter held at 5° C. In four of the fifteen lots proteolytic decomposition was indicated by the development of cheesy flavors. The numbers of proteolytic bacteria in lots which showed cheesy flavor ranged from 2,750,000 to 23,000,000 per ml. at the time the defect was

Table XXI

Comparison of Numbers of Lipolytic and Proteolytic Bacteria and Flavor Defects in Unsalted Butter Held 56 Days at 5° C.

Churning no.	Type of bacteria	0 days	7 days	21 days		28 days		56 days	
		Bacteria per ml.	Bacteria per ml.	Bacteria per ml.	Flavor defect	Bacteria per ml.	Flavor defect	Bacteria per ml.	Flavor defect
1	Lip.	< 100	800	< 1,000		30,000	sl.off	6,000,000	sl. rancid
	Prot.	< 100	< 100	< 1,000		32,000		9,100,000	
2	Lip.	3,500	195,000	150,000	sl. rancid	850,000	rancid	1,750,000	cheesy
	Prot.	2,500	210,000	190,000		750,000		16,000,000	
3	Lip.	< 100	130,000	11,000,000	rancid	8,200,000	cheesy	2,100,000	rancid
	Prot.	< 100	160,000	11,000,000		23,000,000		2,600,000	
4	Lip.	1,000	1,850,000	9,500,000	rancid	1,000,000	rancid	2,000,000	rancid
	Prot.	1,700	1,900,000	18,000,000		2,000,000		1,950,000	
5	Lip.	2,000	22,500	1,350,000	rancid	450,000	rancid	750,000	cheesy, rancid
	Prot.	1,500	30,000	4,300,000		2,100,000		3,200,000	
6	Lip.	250	610,000	1,000,000	sl.off	6,100,000	sl. rancid	1,050,000	rancid
	Prot.	250	35,000	7,000,000		18,500,000		3,600,000	
7	Lip.	200	7,000,000	2,250,000	sl.off	1,250,000	sl. rancid	650,000	sl. rancid
	Prot.	200	1,900,000	2,700,000		3,850,000		650,000	
8	Lip.	< 100	132,000	2,100,000	sl. rancid	2,250,000	sl. rancid	3,150,000	sl. rancid
	Prot.	< 100	132,000	3,400,000		3,200,000		7,500,000	

(continued on following page)

Table XXI (continued)

Comparison of Numbers of Lipolytic and Proteolytic Bacteria and Flavor Defects in Unsalted Butter Held 56 Days at 5° C.

Churning no.	Type of bacteria	0 days	7 days	21 days		28 days		56 days	
		Bacteria per ml.	Bacteria per ml.	Bacteria per ml.	Flavor defect	Bacteria per ml.	Flavor defect	Bacteria per ml.	Flavor defect
9	Lip.	3,600	210,000	390,000	sl.roq-	4,550,000	sl.	4,000,000	rancid
	Prot.	3,800	850,000	1,600,000	uefort	1,600,000	rancid	3,000,000	
10	Lip.	< 100	50,000	1,100,000	sl.	900,000	sl.	120,000	sl.
	Prot.	< 100	< 1,000	< 10,000	rancid	< 10,000	rancid	< 10,000	rancid
11	Lip.	< 100	120,000	150,000	sl.off	210,000	sl.off	3,400,000	rancid
	Prot.	< 100	100,000	400,000		25,000		3,600,000	
12	Lip.	450	< 1,000	250,000		300,000	sl	400,000	roquefort
	Prot.	400	< 1,000	< 10,000		< 10,000	rancid	1,300,000	
13	Lip.	< 100	< 1,000	200,000	sl.off	400,000	sl.off	900,000	roquefort
	Prot.	< 100	650,000	610,000		500,000		1,750,000	
14	Lip.	300	1,000	15,000		30,000	sl.	3,000,000	rancid
	Prot.	< 100	5,000	150,000		140,000	rancid	3,100,000	
15	Lip.	500	40,000	2,300,000	sl.	3,300,000	sl.	2,100,000	rancid
	Prot.	< 100	330,000	3,500,000	rancid	2,750,000	cheesy	690,000	

detected.

Changes in numbers of bacteria and in the flavors of butter held at 0° C.

Table XXII presents the numbers of bacteria in unsalted butter held 56 days at 0° C. The highest average counts on butter held at 0° C. were obtained after 56 days. This general tendency was quite consistent with the various samples, as indicated by the fact that only two of the fifteen lots showed the highest count before the 56 day examination. The ranges of counts were as follows:

Initial 104,000 to 3,140,000 per ml.

After 14 days 65,000 to 11,800,000 per ml.

After 28 days 65,000 to 50,200,000 per ml.

After 56 days 2,900,000 to 146,000,000 per ml.

The results of the total bacterial counts on salted butter held 56 days at 0° C. are presented in Table XXIII. In general, the numbers of bacteria decreased in the salted butter held at 0° C. The lowest counts were found after 56 days at 0° C. and the trend was quite consistent, as indicated by the fact that the lowest counts on thirteen of the fifteen lots occurred at 56 days.

A comparison of the trends of the counts recorded in Tables XXII and XXIII shows the influence of salt on the numbers of bacteria in butter held at 0° C. As indicated by the G. M. of the counts before and after holding, the numbers of bacteria increased thirtyfold in the unsalted butter at 56 days, while the numbers in the corresponding salted butter decreased to about one-fifth of the initial numbers.

Table XXII

Changes in Numbers of Total Bacteria in Unsalted Butter Held 56 Days at 0° C.

Churning no.	Numbers of bacteria per ml.			
	0 days	14,days	28 days	56 days
1	780,000	4,660,000	17,500,000	146,000,000
2	540,000	207,000	690,000	17,800,000
3	3,140,000	3,200,000	6,700,000	60,200,000
4	420,000	11,800,000	48,000,000	114,000,000
5	540,000	10,900,000	8,800,000	20,300,000
6	104,000	65,000	65,000	2,900,000
7	640,000	4,100,000	50,200,000	142,000,000
8	2,030,000	5,300,000	18,100,000	7,900,000
9	2,640,000	5,400,000	22,000,000	11,900,000
10	360,000	380,000	145,000	9,100,000
11	1,180,000	970,000	11,100,000	25,800,000
12	1,530,000	450,000	3,500,000	46,700,000
13	1,620,000	900,000	22,500,000	37,000,000
14	1,330,000	670,000	370,000	22,300,000
15	930,000	2,560,000	23,100,000	27,800,000
Avg.	1,186,000	3,437,000	15,520,000	46,110,000
G. M.	877,000	1,560,000	5,150,000	27,300,000

Table XXIII

Changes in Numbers of Total Bacteria in Salted Butter Held 56 Days at 0° C.

Churning no.	Numbers of bacteria per ml.			
	0 days	14 days	28 days	56 days
1	346,000	98,000	43,000	112,000
2	111,000	56,000	47,000	47,000
3	213,000	43,000	11,900	4,900
4	64,000	56,000	21,400	7,700
5	330,000	7,300	10,600	4,900
6	18,000	37,000	17,500	15,300
7	196,000	198,000	92,000	23,300
8	295,000	312,000	277,000	118,000
9	390,000	243,000	160,000	126,000
10	55,000	54,000	7,700	2,250
11	280,000	113,000	147,000	230,000
12	272,000	218,000	197,000	149,000
13	130,000	120,000	175,000	12,000
14	175,000	210,000	132,000	116,000
15	181,000	173,000	88,000	34,000
Avg.	204,000	129,200	95,100	66,800
G. M.	161,000	92,600	56,200	30,500

The results of lipolytic and proteolytic counts and flavor examinations on unsalted butter held 56 days at 0° C. are presented in Table XXIV. The numbers of lipolytic bacteria increased in fourteen of the fifteen lots of unsalted butter held at 0° C. With one notable exception the samples which contained large numbers of lipolytic bacteria also developed off-flavors. The predominating type of off-flavor was rancidity with nine of the fifteen lots showing such a defect after 56 days. The range of counts of lipolytic bacteria in the lots which developed rancidity was from less than 1,000 to 11,200,000 per ml.

The numbers of proteolytic bacteria increased in fourteen of the fifteen lots of unsalted butter at 0° C. but the flavor defects suggested proteolytic changes in only one lot.

Comparison of the changes in numbers of bacteria and the occurrence of flavor defects in unsalted butter held at different temperatures

In preceding trials the numbers of bacteria in unsalted butter increased at each of the different holding temperatures. The relationship of such bacterial activity to flavor deterioration in unsalted butter is indicated in Table XXV, by a comparison of the trends in counts and the appearance of flavor defects.

A comparison of the trends in numbers of bacteria in unsalted butter at different temperatures indicates that corresponding high points were reached after a longer holding period with each successively lower temperature. These high points do not nescessarily represent the maximum numbers reached at the different temperatures but rather the points at which

Table XXIV

Comparison of Numbers of Lipolytic and Proteolytic Bacteria and Flavor Defects in Unsalted Butter Held 56 Days at 0° C.

Churning no.	Type of Bacteria	0 days	14 days	28 days		56 days	
		Bacteria per ml.	Bacteria per ml.	Bacteria per ml.	Flavor defect	Bacteria per ml.	Flavor defect
1	Lip.	< 100	< 100	46,000	sl. bitter	8,000,000	sl. rancid
	Prot.	< 100	650	50,000		15,500,000	
2	Lip.	3,500	450	78,000		310,000	rancid
	Prot.	2,500	1,200	86,000		3,000,000	
3	Lip.	< 100	420,000	1,500,000	sl. unclean	3,850,000	sl. rancid
	Prot.	< 100	480,000	1,500,000		6,500,000	
4	Lip.	1,000	200,000	4,000,000	rancid	11,200,000	rancid
	Prot.	1,700	590,000	3,900,000		5,600,000	
5	Lip.	2,000	215,000	300,000	sl. unclean	350,000	rancid
	Prot.	1,500	130,000	1,200,000		1,200,000	
6	Lip.	250	< 100	5,400		< 1,000	sl. rancid
	Prot.	250	< 100	5,400		5,000	
7	Lip.	200	70,000	3,300,000	sl. rancid	600,000	sl. rancid
	Prot.	200	120,000	4,900,000		1,100,000	
8	Lip.	< 100	125,000	750,000		35,000	
	Prot.	< 100	120,000	1,450,000		65,000	
9	Lip.	3,600	20,000	3,500,000		80,000	
	Prot.	3,800	20,000	3,500,000		125,000	
10	Lip.	< 100	5,000	< 1,000		750,000	sl. rancid
	Prot.	< 100	5,000	< 1,000		1,450,000	
11	Lip.	< 100	15,000	25,000		900,000	sl. rancid
	Prot.	< 100	20,000	25,000		1,300,000	
12	Lip.	450	500	< 1,000		310,000	sl. cheesy
	Prot.	480	500	40,000		< 10,000	
13	Lip.	< 100	< 100	450,000		2,100,000	sl. off
	Prot.	< 100	5,000	700,000		1,700,000	
14	Lip.	500	< 100	< 1,000		< 10,000	
	Prot.	< 100	< 100	< 1,000		< 10,000	
15	Lip.	500	20,000	400,000		1,350,000	
	Prot.	< 100	100,000	700,000		< 10,000	

Table XXV

A Comparison of the Numbers of Total Bacteria and the Occurrence of Flavor Defects in Unsalted Butter Held at Different Temperatures

Numbers of bacteria expressed as geometric means

Temperature of holding		21° C.	15° C.	5° C.	0° C.
Number of churnings		15	7	15	15
1st examination	Age of butter	0 days	0 days	0 days	0 days
	Bacteria per ml.	877,000	979,000	877,000	877,000
2nd examination	Age of butter	2 days	7 days	7 days	14 days
	Bacteria per ml.	16,540,000	14,600,000	5,590,000	1,560,000
	No. defective lots		three	two	two
3rd examination	Age of butter	4 days	14 days	21 days	28 days
	Bacteria per ml.	20,180,000	34,800,000	29,300,000	5,150,000
	No. defective lots	three	seven	twelve	five
4th examination	Age of butter	7 days	28 days	28 days	56 days
	Bacteria per ml.	20,300,000	25,200,000	28,200,000	27,300,000
	No. defective lots	nine	seven	fifteen	eleven
5th examination	Age of butter			56 days	
	Bacteria per ml.			42,200,000	
	No. defective lots			fifteen	

much.slower growth or actual decreases in numbers were noted. The corresponding high points in counts were as follows:

After 2 days holding at 21° C.

After 7 days holding at 15° C.

After 21 days holding at 5° C.

After 56 days holding at 0° C.

In general, the bacterial counts in unsalted butter increased more rapidly at the higher holding temperatures. The numbers increased most rapidly in butter at 21° C. but the counts failed to reach as high a level within 7 days at 21° C. as was reached in the butter held for longer periods at the lower temperatures, viz. 15°, 5°, and 0° C. Since the upward trend in numbers of bacteria was accelerated with each successive increase in holding temperature, it might be expected that bacteriological deterioration in butter would likewise be accelerated.

There appeared to be a general agreement between the time at which the unsalted butter became defective and the time at which the highest counts were obtained except in the case of the butter held at 21° C. At this temperature, only three of the fifteen lots were defective at 4 days in spite of the fact that the counts were comparatively high at this time.

All of the lots held at 15° C. were defective in flavor within 14 days and the highest count at this temperature was also noted at this time. Twelve of the fifteen lots held at 5° C. were defective in flavor at 21 days and at this time the counts had reached a level corresponding to the highest counts noted at 21° and at 15° C. The greatest number of defective lots were noted in the butter at 0° C. after the 56 day examin-

ation and the bacterial counts were approximately at the same level as those noted at higher temperatures when extensive flavor deterioration had occurred.

The fact that there was a closer agreement between large increases in numbers of total bacteria and the occurrence of flavor defects in butter held at the lower temperatures than in the corresponding butter held at 21° C. suggests that a comparison of the types of bacteria at these different temperatures might offer some explanation. The temperature of 21° C. was favorable to the butter culture types and, no doubt, these types predominated at this temperature and maintained conditions which prevented extensive bacteriological deterioration. The lower temperatures were less favorable to the butter culture types than to the other types such as fat splitting and casein digesting bacteria.

Comparison of numbers of lipolytic and proteolytic bacteria in unsalted butter held at different temperatures

The comparative growth of lipolytic and proteolytic bacteria in unsalted butter held at 21° C. and in butter held at lower temperatures is indicated in Tables XXVI and XXVII.

A summary showing the numbers of lipolytic bacteria in unsalted butter held at different temperatures is given in Table XXVI. The numbers of lipolytic bacteria in the fresh butter were very small as is indicated by the range in counts from less than 100 to 3,600 per ml. The lipolytic bacteria increased in unsalted butter at all of the different temperatures of holding. The extent of the increase is indicated by the range of

Table XXVI

Numbers of Lipolytic Bacteria in Unsalted Butter

Held at Different Temperatures

Temperature of holding	Time of plating	Number of lots	Range in numbers of lipolytic bacteria per ml.	Percentage of lots developing over 1,000,000 per ml.
	0 days	15	< 100 - 3,600	
21° C.	2 days 4 days 7 days	15	< 1,000 - 6,100,000 < 1,000 - 7,400,000 < 1,000 - 12,000,000	46.7
15° C.	7 days 14 days 28 days	7	4,000 - 1,900,000 600,000 - 2,950,000 15,000 - 4,800,000	100
5° C.	7 days 21 days 28 days 56 days	15	< 1,000 - 7,000,000 < 1,000 - 11,000,000 30,000 - 8,200,000 120,000 - 6,000,000	86.7
0° C.	14 days 28 days 56 days	15	< 100 - 420,000 < 1,000 - 4,000,000 < 1,000 - 11,200,000	46.7

counts of lipolytic bacteria and the percentage of the lots in which the numbers of lipolytic bacteria exceeded 1,000,000 per ml. at some time during holding. The numbers of lipolytic bacteria increased more regularly and reached above 1,000,000 per ml. more frequently at 15° and 5° C. than at 21° and 0° C. All of the lots held at 15° C. showed lipolytic bacteria at each period of examination and all contained more than 1,000,000 lipolytic bacteria per ml. at some time during the holding period. At 5° C. all lots showed lipolytic bacteria at the 28 and 56 day examinations and 86.7 per cent of the lots contained more than 1,000,000 lipolytic bacteria per ml. at some time during the holding period. The occurrence of lipolytic bacteria in unsalted butter at 21° C. and 0° C. was much more irregular. In either case the numbers of lipolytic bacteria reached 1,000,000 per ml in 46.7 per cent of the lots.

A summary of the numbers of proteolytic bacteria occurring in unsalted butter held at different temperatures is given in Table XXVII. The numbers of proteolytic bacteria in the fresh butter were very low, as indicated by the range in counts of from less than 100 to 3,800 per ml. The proteolytic bacteria increased in unsalted butter held at each of the different temperatures. The most extensive increase was noted in the butter held at 5° C. as indicated by the generally higher range of counts and the fact that 93.3 per cent of the lots contained over 1,000,000 proteolytic bacteria per ml. at some time during the holding period. All lots of butter held at 15° C. showed proteolytic bacteria at the 14 and 28 day examinations, and 71.4 per cent of the lots developed more than 1,000,000 proteolytic bacteria per ml. The numbers of proteo-

Table XXVII

Numbers of Proteolytic Bacteria in Unsalted Butter

Held at Different Temperatures

Temperature of holding	Time of plating	Number of lots	Range in numbers of proteolytic bacteria per ml.	Percentage of lots developing over 1,000,000 per ml.
	0 days	15	100 - 3,800	
21° C.	2 days 4 days 7 days	15	1,000 - 2,500,000 1,000 - 2,750,000 10,000 - 3,500,000	40
15° C.	7 days 14 days 28 days	7	1,000 - 1,100,000 500,000 - 1,500,000 15,000 - 8,500,000	71.4
5° C.	7 days 21 days 28 days 56 days	15	100 - 1,900,000 10,000 - 18,000,000 10,000 - 23,000,000 10,000 - 16,000,000	93.3
0° C.	14 days 28 days 56 days	15	100 - 590,000 1,000 - 4,900,000 10,000 - 15,500,000	73.3

lytic bacteria increased to more than 1,000,000 per ml. in 73.3 per cent of the lots held at 0° C. and in 40 per cent of the lots held at 21° C.

In general, the numbers of lipolytic and proteolytic bacteria increased more extensively and reached counts above 1,000,000 per ml. more frequently in unsalted butter held at 5° C. than in the butter at either higher or lower temperatures.

Changes in numbers of total bacteria in salted butter held at different temperatures

In the preceding trials the numbers of bacteria in salted butter decreased at each of the different holding temperatures. A comparison of the trends in counts at the different temperatures is presented in Table XXVIII. Corresponding low points in the trends were noted after a longer holding period with each successively lower temperature. These low points do not represent the lowest counts obtained on salted butter at each temperature but rather the points at which definite changes in trends occurred. Corresponding low points in the trends of bacterial counts were recorded as follows:

After 4 days at 21° C.

After 7 days at 15° C.

After 21 days at 5° C.

After 28 days at 0° C.

It may be noted that the times at which corresponding low points were observed with salted butter coincide fairly well with the times at which high points were observed with the counts on unsalted butter.

Table XXVIII

Changes in Numbers of Total Bacteria in Salted Butter Held at Different Temperatures

Numbers of bacteria expressed as geometric means

Temperature of holding		21° C.	15° C.	5° C.	0° C.
Number of churnings		15	7	15	15
1st examination	Age of butter	0 days	0 days	0 days	0 days
	Bacteria per ml.	161,000	183,000	161,000	161,000
2nd examination	Age of butter	2 days	7 days	7 days	14 days
	Bacteria per ml.	88,000	54,000	88,800	92,600
3rd examination	Age of butter	4 days	14 days	21 days	28 days
	Bacteria per ml.	53,100	59,100	46,400	56,200
4th examination	Age of butter	7 days	28 days	28 days	56 days
	Bacteria per ml.	51,000	72,600	37,400	30,500
5th examination	Age of butter			56 days	
	Bacteria per ml.			26,200	

The decrease in counts was more rapid with the higher temperatures; however, the two lowest counts resulted after holding at the two lowest temperatures. Whether or not the reduction in numbers of bacteria would have continued with longer holding periods at 21° and 15° C. is doubtful. The counts at 21° C. failed to show significant reduction after 4 days, while the counts on butter held at 15° C. appeared to increase slightly toward the end of the 28 day period.

Time of appearance and nature of flavor defects in unsalted butter held at different temperatures

The flavor defects appearing in the unsalted butter of churnings 1 to 15 inclusive have been reported in previous tables for comparison with the occurrence of lipolytic and proteolytic bacteria. Table XXIX gives a summary of the flavor defects occurring in the unsalted butter held at different temperatures. The periods at which flavor examinations were made were the same as those used in making the bacteriological examination reported in previous tables. The data on each sample includes the defect first noted and the final defect developed during holding at each temperature.

For the purpose of showing the value of holding butter at 21° C. for the prediction of keeping qualities at the lower temperatures, the following comparisons are presented. Seven lots of unsalted butter held at 15° C. showed flavor defects after 14 days and only three of these lots developed flavor defects within 7 days at 21° C. Fifteen lots of unsalted butter held at 5° C. showed flavor defects after periods varying from 21

Table XXIX

Comparison of Time of Appearance and Nature of Flavor Defects in Unsalted Butter Held at Different Temperatures

Churning no.	Flavor comments on butter held at							
	21° C.		15° C.		5° C.		0° C.	
	Days	Flavor defect	Days	Flavor defect	Days	Flavor defect	Days	Flavor defect
1	7	---			28 56	sl. off sl.rancid	28 56	sl.bitter sl.rancid
2	7	---			21 28	sl.rancid rancid	56	rancid
3	7	sl. off			21 28	rancid cheesy	28 56	unclean sl.rancid
4	4 7	sl.rancid rancid			21 28	rancid rancid	28 56	rancid rancid
5	7	rancid			21 56	rancid cheesy,rancid	28 56	sl.unclean rancid
6	7	sl. off			21 28	sl. off sl.rancid	56	sl.rancid
7	7	sour			21 28	sl. off sl.rancid	28 56	sl.rancid sl.rancid
8	7	sl. off			21 28	sl.rancid sl.rancid	56	---
9	7	---	14	sl.roquefort	21 56	sl.roquefort rancid	56	---
10	7	---	14	sl.rancid	21 56	sl.rancid sl.rancid	56	sl.rancid
11	7	sl. off	7 14	sl. off sl.rancid	21 56	sl. off rancid	56	sl.rancid
12	4 7	sl. off sl.rancid	7 14	sl. off roquefort	28 56	sl. rancid roquefort	56	sl.cheesy
13	7	---	7 14	sl. off sl. off	21 56	sl. off roquefort	56	sl. off
14	4 7	sour sour	14	sl.rancid	28 56	sl.rancid, metallic rancid	56	---
15	7	---	14	sl. off	21 28	sl.rancid sl.cheesy	56	---

--- Flavor satisfactory
sl. = slightly

to 28 days and only nine of these lots showed flavor defects within 7 days at 21° C. Eleven lots of unsalted butter held at 0° C. showed flavor defects within the 56 day period and only seven of these lots showed flavor defects within 7 days at 21° C. The off-flavors which were noted in the unsalted butter at 21° C. were generally of a milder and less definite nature than those noted at lower temperatures. Furthermore, the growth of butter culture organisms imparted a definite ripened flavor which may have masked the incipient stages of specific flavor defects.

The results in Table XXIX suggest that a longer period of observation at 21° C. might give more accurate information on the keeping quality of unsalted butter held at lower temperatures. Flavor defects did not appear within 7 days at 21° C. in certain lots when the corresponding lots held at lower temperatures showed pronounced flavor deterioration. Since the off-flavors developed in the unsalted butter held at 21° C. were frequently of a mild and indefinite nature, it appeared possible that defects in certain lots may have been overlooked.

The trial reported in Table XXX was carried out to show the value of an extended period of observation at 21° C. for the prediction of keeping quality at lower temperatures. A new series of ten churnings of unsalted butter were sampled and one lot from each churning was placed at each of the different temperatures. The periods of examination were as follows:

4, 7, and 10 days at 21° C.

4, 7, 10, and 14 days at 15° C.

7, 14, 21, 28, and 60 days at 5° C.

14, 21, 28, and 60 days at 0° C.

Table XXX

Comparison of Time of Appearance and Nature of Flavor Defects in Unsalted Butter Held at Different Temperatures

Churning no.	Flavor comments on butter held at							
	21° C.		15° C.		5° C.		0° C.	
	Days	Flavor defect	Days	Flavor defect	Days	Flavor defect	Days	Flavor defect
16	10	---	14	sl. fruity	28	sl. rancid	60	sl. rancid
17	7	cheesy	10 14	sl. cheesy cheesy	14 21	sl. cheesy cheesy	14 28	sl. woody bitter, cheesy
18	10	---	14	---	60	---	60	---
19	10	---	14	---	60	---	60	---
20	10	rancid	10 14	sl. rancid oily, rancid	14 21	sl. rancid sl. rancid	60	---
21	4 7	sl. cheesy sl. cheesy	7 10	sl. off sl. cheesy	10 28	sl. off cheesy	21 60	sl. cheesy cheesy
22	10	---	14	---	60	---	60	---
23	10	---	14	sl. off	21 28	sl. rancid roquefort	60	---
24	4 10	sour sl. cheesy	10 14	stale sl. cheesy	14 28	unclean cheesy	60	sl. cheesy
25	10	sour	10 14	sl. off sour	28	sour	60	rancid

--- Flavor satisfactory

sl. = slightly

The data recorded in Table XXX include the flavor defects first noted and the final defect noted during the period of holding at each temperature. The seven lots of unsalted butter held at 15° C. showed flavor defects after 14 days and five of the corresponding lots became defective within 10 days at 21° C. Seven of the lots held at 5° C. showed flavor defects after 21 to 28 days and five of the corresponding lots held at 21° C. became defective within 10 days. Five of the lots held at 0° C. showed flavor defects at the 60 day examination and four of the corresponding lots became defective within 10 days when held at 21° C. The butter which deteriorated at 21° C. frequently showed the same flavor defects that were found in the corresponding butter after holding at lower temperatures. The extension of the period of observation to 10 days at 21° C. evidently gave added information on keeping quality as indicated by the closer agreement of the results at 21° C. with the results at lower temperatures. In three of the five lots which deteriorated when held at 21° C., the flavor defect was not detected until the 10 day examination. This indicates that flavor deterioration in unsalted butter held at 21° C. occurred too slowly to be detected regularly within 7 days.

When portions of a churning of unsalted butter showed flavor defects at 5° C. and 0° C. the corresponding butter became defective within 14 days at 15° C. but in two cases did not develop defects within 10 days at 21° C. In the portions which deteriorated at both 15° and 21° C., however, the defects required more time for development at 15° C. than at 21° C. The close agreement of flavor deterioration at 15° C. with deterioration at lower temperatures indicates that the bacterial action in

butter at 15° C. compared more closely with the action of bacteria in butter at lower holding temperatures than did the bacterial action at 21° C. This relationship has been indicated previously in the comparison of changes in numbers of lipolytic and proteolytic bacteria in butter held at the different temperatures.

SECTION B

FLAVOR DETERIORATION AND BACTERIOLOGICAL CHANGES IN BUTTER MADE WITHOUT BUTTER CULTURE AND HELD AT DIFFERENT TEMPERATURES

The trials in Section B were planned to give additional information on the comparative periods required for flavor deterioration and on the changes in numbers of bacteria in butter held at different temperatures. The plan of investigation was similar to that used in Part III except that non-culture butter was used. The butter was obtained from the butter laboratory at South Dakota State College. The cream for churning was received from producers in the vicinity of the plant and was of relatively good quality. Twenty-four of the thirty-four churnings used in this study were made from cream of less than 0.35 per cent acidity and none of the churnings involved cream containing in excess of 0.60 per cent acidity. The trials included butter made over a period extending from September to February.

Included in Section B were 16 sets of samples taken from churnings made without butter culture. Two samples from each set, one salted and one unsalted, were placed at each temperature and studies were made of the flavor deterioration and of the changes in numbers of total, lipolytic, and proteolytic bacteria. Eighteen additional churnings of unsalted, non-culture butter were studied for flavor deterioration only. These churnings differed from the first group only in the time at which they were made. They were made over a period extending from December 1

to February 1 while the first group was made between September 1 and December 1.

Changes in numbers of bacteria and in the flavor of butter held 7 days at 21° to 26° C.

Table XXXI presents the bacterial counts on the unsalted butter held 7 days at 21° to 26° C. The average numbers of bacteria increased during the first 4 days and then decreased. The highest counts were obtained after 2 days with five lots, after 4 days with five lots, and after 7 days with six lots. The most significant increase occurred during the first two days of the holding period. The ranges of counts were as follows:

Initial counts 16,000 to 2,120,000 per ml.

After 2 days 4,300,000 to 76,300,000 per ml.

After 4 days 3,800,000 to 211,000,000 per ml.

After 7 days 5,300,000 to 101,000,000 per ml.

A comparison of the changes in numbers of bacteria in this non-culture butter with the changes in counts in the culture butter reported in Table XIII indicates that the increases were much more extensive in the non-culture butter held at 21° to 26° C. than in culture butter held at 21° C. The non-culture butter increased in counts more than one hundredfold, while the culture butter increased about twenty-threefold.

The total bacterial counts on the salted butter held 7 days at 21° to 26° C. are presented in Table XXXII. In direct contrast to the results in Table XIV on the salted butter containing butter culture, the number of bacteria in the non-culture, salted butter increased somewhat at 21° to 26° C. Thirteen of sixteen lots contained greater numbers of bacteria

Table XXXI

Changes in Numbers of Total Bacteria in Unsalted Butter

Held 7 Days at 21° to 26° C.

Churning no.	Numbers of bacteria per ml.			
	0 days	2 days	4 days	7 days
26	16,000	41,300,000	211,000,000	78,000,000
27	97,000	76,300,000	144,000,000	73,000,000
28	175,000	28,300,000	23,000,000	9,900,000
29	147,000	13,900,000	12,900,000	9,100,000
30	103,000	53,000,000	34,000,000	54,000,000
31	2,120,000	17,000,000	16,600,000	64,000,000
32	446,000	4,300,000	149,000,000	45,000,000
33	350,000	---	79,000,000	58,000,000
34	830,000	17,300,000	18,500,000	27,500,000
35	990,000	72,000,000	70,000,000	38,800,000
36	760,000	5,900,000	3,800,000	5,300,000
37	760,000	34,100,000	37,000,000	41,000,000
38	1,390,000	49,000,000	54,500,000	80,000,000
39	910,000	47,000,000	36,000,000	101,000,000
40	160,000	66,000,000	53,000,000	35,400,000
41	90,000	11,200,000	22,000,000	20,800,000
Avg.	584,000	35,800,000	60,300,000	46,300,000
G. M.	315,000	26,300,000	38,700,000	35,500,000

--- Sample missed

Table XXXII

Changes in Numbers of Total Bacteria in Salted Butter

Held 7 Days at 21° to 26° C.

Churning no.	Numbers of bacteria per ml.			
	0 days	2 days	4 days	7 days
26	11,000	101,000	354,000	2,300,000
27	87,000	407,000	470,000	784,000
28	179,000	327,000	1,960,000	922,000
29	101,000	66,000	102,000	280,000
30	134,000	47,000	30,000	257,000
31	123,000	108,000	129,000	874,000
32	178,000	22,000	970,000	900,000
33	174,000	---	125,000	858,000
34	158,000	125,000	550,000	348,000
35	151,000	260,000	880,000	2,730,000
36	170,000	89,000	104,000	112,000
37	142,000	106,000	660,000	1,930,000
38	26,000	147,000	902,000	1,440,000
39	239,000	55,000	53,000	90,000
40	68,000	44,000	86,000	313,000
41	42,000	42,000	3,900	6,900
Avg.	124,000	130,000	461,000	884,000
G. M.	115,000	129,000	203,000	464,000

--- Sample missed

after 7 days than were present at the initial examination. The results indicate that the butter culture organisms in the salted butter included in Table XIV were more succeptible to the destructive action of salt than the natural flora of the butter included in Table XXXII.

A comparison of the results in Tables XXXI and XXXII shows the influence of salt on the numbers of bacteria in butter held at 21° to 26° C. As indicated by the G. M. of the counts before and after holding, the numbers of bacteria in unsalted butter increased approximately one hundredfold while the numbers in salted butter increased about fourfold.

The numbers of lipolytic and proteolytic bacteria and the flavor defects developed in the unsalted butter held 7 days at 21° to 26° C. are presented in Table XXXIII. Lipolytic bacteria were found in only three of the sixteen lots of fresh butter and in these the numbers did not exceed 500 per ml. The numbers increased in nine of the sixteen lots but counts of over 1,000,000 per ml. were noted on only two lots. There was no close correlation between the occurrence of lipolytic bacteria and the development of rancid or roquefort flavors.

Proteolytic bacteria were found in seven of the sixteen lots of unsalted butter at the initial examination and the numbers in these seven lots ranged from 200 to 25,000 per ml. The numbers of proteolytic bacteria increased in fourteen of the sixteen lots and reached counts of over 1,000,000 per ml. in eight lots. In general, the lots with high counts of proteolytic bacteria showed flavor deterioration within 7 days but the flavor defects indicated proteolysis in only three instances. The highest count of proteolytic bacteria was obtained on churning 39

Table XXXIII

Comparison of Numbers of Lipolytic and Proteolytic Bacteria and Flavor Defects in Unsalted Butter Held 7 Days at 21° to 26° C.

Churning no.	Type of bacteria	0 days	2 days		4 days		7 days	
		Bacteria per ml.	Bacteria per ml.	Flavor defect	Bacteria per ml.	Flavor defect	Bacteria per ml.	Flavor defect
26	Lip. Prot.	500 3,000	< 10,000 900,000		< 10,000 61,000,000	roquefort	< 10,000 9,000,000	roquefort
27	Lip. Prot.	< 100 92,000	< 10,000 10,900,000		< 10,000 17,500,000	roquefort	< 10,000 9,000,000	roquefort
28	Lip. Prot.	< 100 < 100	< 10,000 35,000		< 10,000 850,000		< 10,000 1,250,000	
29	Lip. Prot.	< 100 2,000	< 10,000 < 10,000		< 10,000 < 10,000		< 10,000 < 10,000	fermented
30	Lip. Prot.	< 100 < 100	< 10,000 2,500,000		< 10,000 7,500,000		< 10,000 2,300,000	rancid
31	Lip. Prot.	< 100 200	< 10,000 63,000	sour	< 10,000 230,000	sour	500,000 3,100,000	cheesy
32	Lip. Prot.	400 400	50,000 50,000		100,000 200,000		< 10,000 800,000	rancid
33	Lip. Prot.	< 100 < 100	--- ---		< 10,000 < 10,000		< 10,000 < 10,000	sl.cheesy

(table continued on following page)

Table XXXIII (continued)

Comparison of Numbers of Lipolytic and Proteolytic Bacteria and Flavor Defects in Unsalted Butter Held 7 Days at 21° to 26° C.

Churning no.	Type of bacteria	0 days	2 days		4 days		7 days	
		Bacteria per ml.	Bacteria per ml.	Flavor defect	Bacteria per ml.	Flavor defect	Bacteria per ml.	Flavor defect
34	Lip. Prot.	500 200	55,000 30,000	sour	800,000 < 10,000	roquefort	20,000 < 10,000	roquefort
35	Lip. Prot.	< 100 < 100	1,350,000 1,300,000	sour	1,200,000 1,200,000	sl. rancid	1,950,000 2,200,000	roquefort
36	Lip. Prot.	< 100 < 100	< 10,000 < 10,000		< 10,000 < 10,000		20,000 20,000	
37	Lip. Prot.	< 100 < 100	< 10,000 7,000		< 10,000 < 10,000		< 10,000 < 10,000	
38	Lip. Prot.	< 100 < 100	55,000 20,000		100,000 250,000		100,000 700,000	
39	Lip. Prot.	< 100 25,000	400,000 2,700,000		1,200,000 2,050,000	sour	500,000 16,000,000	cheesy
40	Lip. Prot.	< 100 < 100	< 10,000 < 10,000		< 10,000 70,000		400,000 800,000	
41	Lip. Prot.	< 100 < 100	< 10,000 1,200,000		200,000 300,000	sour	< 10,000 800,000	

--- Sample missed

which contained 16,000,000 proteolytic bacteria per ml. at the time that the cheesy flavor was detected.

Changes in numbers of bacteria and in flavor of butter held 56 days at 5°C.

Table XXXIV presents the total bacterial counts on the unsalted butter held 56 days at 5° C. In general, the numbers of bacteria increased but the time at which the highest counts were obtained varied with different lots. The maximum numbers were recorded at 7 days with one lot, at 14 days with one lot, at 28 days with six lots, and at 56 days with eight lots.

The ranges of counts were as follows:

Initial 16,000 to 2,120,000 per ml.

After 7 days 30,000 to 55,000,000 per ml.

After 14 days 151,000 to 130,000,000 per ml.

After 28 days 2,270,000 to 162,000,000 per ml.

After 56 days 4,700,000 to 254,000,000 per ml.

A comparison of the increase in the bacterial counts on this non-culture, unsalted butter with the increase in the bacterial counts on the butter made with butter culture in Table XIX indicates that the numbers of bacteria in the non-culture butter increased about one hundred-fifty-fold, while the numbers of bacteria in the culture butter held under the same conditions increased about fiftyfold.

The results reported in Table XXXV show the changes in total bacterial counts on the salted butter held 56 days at 5° C. The numbers of bacteria generally decreased and in fourteen of the sixteen lots, lower counts were obtained at 56 days than were obtained at the initial exam-

Table XXXIV

Changes in Numbers of Total Bacteria in Unsalted Butter

Held 56 Days at 5° C.

Churning	Numbers of bacteria per ml.				
no.	0 days	7 days	14 days	28 days	56 days
26	16,000	30,000	5,780,000	18,600,000	35,000,000
27	97,000	32,000	2,580,000	18,000,000	10,200,000
28	175,000	45,000	859,000	61,000,000	43,000,000
29	147,000	143,000	151,000	2,270,000	4,700,000
30	103,000	150,000	21,600,000	54,000,000	254,000,000
31	2,120,000	329,000	15,200,000	62,000,000	90,000,000
32	446,000	390,000	24,000,000	76,000,000	129,000,000
33	350,000	890,000	3,360,000	82,000,000	53,000,000
34	830,000	---	840,000	66,000,000	85,000,000
35	990,000	1,750,000	19,000,000	159,000,000	57,000,000
36	760,000	3,200,000	11,200,000	103,000,000	125,000,000
37	760,000	580,000	42,000,000	43,200,000	72,000,000
38	1,390,000	305,000	29,400,000	162,000,000	79,000,000
39	910,000	55,000,000	41,000,000	51,000,000	20,600,000
40	160,000	1,510,000	130,000,000	81,000,000	75,000,000
41	90,000	1,420,000	40,000,000	66,000,000	33,000,000
Avg.	584,000	4,385,000	24,186,000	69,070,000	73,200,000
G. M.	315,000	471,000	9,150,000	50,840,000	51,130,000

--- Sample missed

Table XXXV

Changes in Numbers of Total Bacteria in Salted Butter

Held 56 Days at 5° C.

Churning no.	Numbers of bacteria per ml.				
	0 days	7 days	14 days	28 days	56 days
26	11,000	3,900	7,100	3,700	3,700
27	87,000	46,000	82,000	41,000	5,000
28	179,000	33,000	92,000	68,000	43,000
29	101,000	72,000	120,000	29,000	59,000
30	134,000	61,000	46,000	39,000	112,000
31	123,000	31,000	58,000	90,000	9,000
32	178,000	93,000	91,000	14,400	14,600
33	174,000	161,000	46,000	58,000	55,800
34	158,000	---	116,000	108,000	2,100
35	151,000	144,000	81,000	155,000	259,000
36	170,000	103,000	101,000	49,000	4,200
37	142,000	159,000	192,000	274,000	132,000
38	26,000	377,000	458,000	270,000	280,000
39	239,000	104,000	130,000	92,000	9,900
40	68,000	70,000	8,000	4,500	2,500
41	42,000	3,800	6,900	5,600	3,800
Avg.	124,000	97,400	102,200	81,300	62,200
G. M.	115,000	58,200	61,600	42,700	19,100

--- Sample missed

ination.

A comparison of the results in Table XXXV with those on the corresponding unsalted butter in Table XXXIV shows the influence of salt on the changes in numbers of bacteria in butter. As indicated by the G. M. of counts before and after holding, the bacterial counts on unsalted butter increased approximately one hundred-fiftyfold, while the bacterial counts on the corresponding salted butter decreased to about one-sixth of the initial numbers.

Table XXXVI presents the numbers of lipolytic and proteolytic bacteria in unsalted butter held 56 days at 5° C. The numbers of lipolytic bacteria increased in thirteen of the sixteen lots and in all of these lots the counts were above 1,000,000 per ml. at some time during the 56 day period. The lots containing large numbers of lipolytic bacteria commonly showed flavor defects at the 28 day examination and the most common defect developed was rancidity. The lots which developed rancid flavor contained from less than 1,000 to 20,000,000 lipolytic bacteria per ml. at the time that rancidity was first noted.

The numbers of proteolytic bacteria increased in fourteen of the sixteen lots of unsalted butter at 5° C. and in all of these lots the counts exceeded 1,000,000 per ml. at some time during the holding period. The flavor defects noted at 56 days very frequently suggested proteolytic activity. The lots which developed cheesy flavors contained from less than 10,000 to 30,000,000 proteolytic bacteria per ml.

The most marked increases in numbers of lipolytic and proteolytic bacteria occurred up to the 28 day examination, concurrently with the development of definite flavor defects. In most of the lots held at 5° C.,

Table XXXVI

Comparison of Numbers of Lipolytic and Proteolytic Bacteria and Flavor Defects in Unsalted Butter Held 56 Days at 5° C.

Churning no.	Type of bacteria	0 days	7 days	14 days		28 days		56 days	
		Bacteria per ml.	Bacteria per ml.	Bacteria per ml.	Flavor defect	Bacteria per ml.	Flavor defect	Bacteria per ml.	Flavor defect
26	Lip.	500	< 100	400,000		1,150,000	sl.rancid	3,300,000	rancid, fruity
	Prot.	3,000	< 100	440,000		1,950,000		4,100,000	
27	Lip.	< 100	< 100	350,000	sl.unclean	4,600,000	cheesy, sl.rancid	1,100,000	cheesy, rancid
	Prot.	92,000	850	420,000		4,400,000		1,200,000	
28	Lip.	< 100	1,450	59,000		7,400,000	sl.rancid	4,100,000	sl.cheesy
	Prot.	< 100	1,900	59,000		10,500,000		3,550,000	
29	Lip.	< 100	< 100	< 1,000		< 10,000		< 10,000	sl.rancid
	Prot.	2,000	< 100	< 1,000		< 10,000		< 10,000	
30	Lip.	< 100	< 100	< 1,000	sl.rancid	20,000,000	rancid, fruity	30,000,000	cheesy
	Prot.	< 100	< 100	750,000		1,200,000		30,000,000	
31	Lip.	< 100	< 100	< 1,000		400,000	unclean	5,500,000	rancid, roquefort
	Prot.	200	< 100	640,000		450,000		3,500,000	
32	Lip.	400	< 100	< 1,000		< 10,000		< 10,000	cheesy, putrid
	Prot.	400	< 100	< 1,000		< 10,000		< 10,000	
33	Lip.	< 100	3,500	410,000		3,500,000	fruity, rancid	1,500,000	cheesy, putrid
	Prot.	< 100	1,500	520,000		16,000,000		3,900,000	
34	Lip.	500	---	80,000		12,000,000	rancid	10,000,000	cheesy, rancid
	Prot.	200	---	110,000		12,000,000		23,000,000	

(continued on following page)

Table XXXVI (continued)

Comparison of Numbers of Lipolytic and Proteolytic Bacteria and Flavor Defects in Unsalted Butter Held 56 Days at 5° C.

Churning no.	Type of bacteria	0 days	7 days	14 days		28 days		56 days	
		Bacteria per ml.	Bacteria per ml.	Bacteria per ml.	Flavor defect	Bacteria per ml.	Flavor defect	Bacteria per ml.	Flavor defect
35	Lip.	< 100	30,000	< 1,000		200,000	fruity, rancid	2,100,000	cheesy
	Prot.	< 100	115,000	500,000		900,000		2,500,000	
36	Lip.	< 100	55,000	1,650,000		4,000,000	fruity, rancid	< 10,000	cheesy
	Prot.	< 100	55,000	2,200,000		14,500,000		28,000,000	
37	Lip.	< 100	< 100	6,000,000		4,000,000	cheesy	3,000,000	fruity, rancid
	Prot.	< 100	< 100	9,500,000		3,800,000		6,500,000	
38	Lip.	< 100	20,000	1,800,000		< 10,000	sl.cheesy	200,000	cheesy
	Prot.	< 100	25,000	2,900,000		11,000,000		3,100,000	
39	Lip.	< 100	1,800,000	2,000,000		2,500,000	rancid, cheesy	750,000	rancid, cheesy
	Prot.	25,000	2,650,000*	3,100,000	cheesy	14,500,000		2,200,000	
40	Lip.	< 100	< 100	< 1,000	sl.cheesy	< 10,000	sl.cheesy, unclean	< 10,000	cheesy
	Prot.	< 100	9,500	6,450,000		17,000,000		11,500,000	
41	Lip.	< 100	< 100	5,500,000	rancid	6,500,000	cheesy	2,500,000	cheesy
	Prot.	< 100	85,000	16,500,000		16,500,000		4,000,000	

--- Sample missed
* cheesy

high counts of either type were rather closely associated with the development of rancid or cheesy flavors. In certain lots, however, rancid or cheesy flavors developed without lipolytic or proteolytic bacteria being detected.

Changes in numbers of bacteria and in flavor defects of butter held 56 days at 0° C.

The total bacterial counts on the unsalted butter held 56 days at 0° C. are given in Table XXXVII. The bacterial counts increased in all of the lots at 0° C. and reached surprisingly large numbers at 56 days. In fifteen of the sixteen lots the counts were higher after 56 days than at any previous examination. The counts had the following ranges:

Initial 16,000 to 2,120,000 per ml.

14 days 40,000 to 38,000,000 per ml.

28 days 1,970,000 to 85,000,000 per ml.

56 days 4,500,000 to 233,000,000 per ml.

A comparison of the increases in numbers of bacteria in this unsalted, non-culture butter with the increases in that made with butter culture in Table XXII indicates that the numbers in the non-culture butter increased about two hundredfold, while the numbers of bacteria in the culture butter held under the same conditions, increased about thirtyfold.

Table XXXVIII presents the total bacterial counts on the salted butter held 56 days at 0° C. In general, a marked decrease in numbers of bacteria occurred in the salted butter. The lowest average count was obtained after 56 days when fourteen of the sixteen lots contained smaller

Table XXXVII

Changes in Numbers of Total Bacteria in Unsalted Butter

Held 56 Days at 0° C.

Churning no.	Numbers of bacteria per ml.			
	0 days	14 days	28 days	56 days
26	16,000	780,000	42,300,000	103,000,000
27	97,000	147,900	8,800,000	12,200,000
28	175,000	97,000	53,000,000	40,900,000
29	147,000	77,000	1,970,000	4,500,000
30	103,000	48,000	77,900,000	233,000,000
31	2,120,000	3,650,000	56,000,000	69,000,000
32	446,000	21,300,000	68,600,000	132,000,000
33	350,000	2,400,000	31,600,000	61,000,000
34	830,000	980,000	20,200,000	122,000,000
35	990,000	10,900,000	85,000,000	120,000,000
36	760,000	5,400,000	64,000,000	208,000,000
37	760,000	27,000,000	26,500,000	128,000,000
38	1,390,000	700,000	75,000,000	80,000,000
39	910,000	38,000,000	41,000,000	59,000,000
40	160,000	40,000	35,000,000	181,000,000
41	90,000	730,000	45,000,000	49,000,000
Avg.	584,000	7,015,500	45,740,000	100,160,000
G. M.	315,400	1,150,000	35,060,000	71,710,000

Table XXXVIII

Changes in Numbers of Total Bacteria in Salted Butter

Held 56 Days at 0° C.

Churning no.	Numbers of bacteria per ml.			
	0 days	14 days	28 days	56 days
26	11,000	5,500	13,700	6,100
27	87,000	66,000	62,000	206,000
28	179,000	60,000	277,000	60,000
29	101,000	150,000	153,000	62,000
30	134,000	46,000	12,000	65,000
31	123,000	66,000	9,900	6,300
32	178,000	137,000	42,000	15,000
33	174,000	76,000	7,500	30,400
34	158,000	85,000	45,000	1,700
35	151,000	127,000	181,000	48,000
36	170,000	74,000	44,000	9,700
37	142,000	97,000	12,800	13,200
38	26,000	426,000	225,000	206,000
39	239,000	124,000	63,000	15,200
40	68,000	34,000	5,200	1,700
41	42,000	2,000	8,600	3,400
Avg.	124,000	98,500	72,600	46,900
G. M.	115,000	60,100	34,400	18,300

numbers of bacteria than were found in the fresh butter.

A comparison of the results in Tables XXXVII and XXXVIII shows the influence of salt on the changes in numbers of bacteria in butter held at 0° C. The G. M. of counts before and after holding show that the numbers of bacteria in unsalted butter increased more than two hundredfold, while the numbers in salted butter were reduced to about one-sixth of the initial number.

Table XXXIX presents the numbers of lipolytic and proteolytic bacteria in the unsalted butter held 56 days at 0° C. The numbers of lipolytic bacteria increased in twelve of the sixteen lots and in nine of these lots the numbers exceeded 1,000,000 per ml. Rancid flavor was noted in eight lots of butter at 28 days and five of these contained large numbers of lipolytic bacteria.

The numbers of proteolytic bacteria increased in fourteen of the sixteen lots and counts of over 1,000,000 per ml. were recorded in these fourteen lots at 28 days and at 56 days. The flavor defects at 56 days frequently suggested proteolytic action. The lots which developed cheesy flavors contained from less than 10,000 to 26,000,000 per ml. at the time that the cheesy flavor was detected. Proteolytic bacteria were not detected in lot No. 32, even though the sample was replated after the 56 day examination when a pronounced cheesy flavor was noted.

The most marked increases in numbers of lipolytic and proteolytic bacteria occurred previous to the 28 day examination, although the numbers increased very extensively between the 28 day and the 56 day examinations. The development of flavor defects was frequently accompanied

Table XXXIX

Comparison of Numbers of Lipolytic and Proteolytic Bacteria and Flavor Defects in Unsalted Butter Held 56 Days at 0° C.

Churning no.	Type of bacteria	0 days	14 days	28 days		56 days	
		Bacteria per ml.	Bacteria per ml.	Bacteria per ml.	Flavor defect	Bacteria per ml.	Flavor defect
26	Lip.	500	< 100	1,500,000	sl. rancid	23,000,000	rancid, cheesy
	Prot.	3,000	< 100	2,500,000		26,000,000	
27	Lip.	< 100	200	1,700,000	sl. cheesy	11,000,000	cheesy
	Prot.	92,000	400	2,150,000		11,000,000	
28	Lip.	< 100	< 100	18,000,000	sl. rancid	6,700,000	sl.rancid
	Prot.	< 100	< 100	16,000,000		6,700,000	
29	Lip.	< 100	< 100	< 10,000		< 10,000	sl.cheesy
	Prot.	2,000	< 100	< 10,000		< 10,000	
30	Lip.	< 100	< 100	4,000,000	sl. rancid	4,500,000	fruity, cheesy
	Prot.	< 100	< 100	9,000,000		17,500,000	
31	Lip.	< 100	<1,000	10,000	sl.off	1,050,000	sl.rancid
	Prot.	200	xxx	2,600,000		1,100,000	
32	Lip.	400	<1,000	< 10,000		< 10,000	cheesy
	Prot.	400	<1,000	< 10,000		< 10,000	
33	Lip.	< 100	10,000	5,100,000	sl. rancid	3,200,000	cheesy
	Prot.	< 100	140,000	6,300,000		3,650,000	
34	Lip.	500	< 1,000	3,100,000	sl. rancid	9,000,000	sl. cheesy
	Prot.	200	50,000	3,100,000		16,000,000	
35	Lip.	< 100	< 1,000	< 10,000	sl. rancid	< 10,000	cheesy
	Prot.	< 100	1,850,000	10,000,000		16,000,000	
36	Lip.	< 100	700,000	< 10,000	sl. rancid	< 10,000	cheesy
	Prot.	< 100	900,000	7,000,000		20,000,000	
37	Lip.	< 100	570,000	450,000	sl.off	11,000,000	fruity, rancid
	Prot.	< 100	520,000	2,300,000		16,000,000	
38	Lip.	< 100	14,000	< 10,000	sl. cheesy	450,000	cheesy
	Prot.	< 100	12,000	11,000,000		5,150,000	
39	Lip.	< 100	< 1,000	< 10,000	sl. rancid	200,000	roquefort
	Prot.	25,000	4,000,000*	4,200,000		2,100,000	
40	Lip.	< 100	< 1,000	< 10,000	sl.off	< 10,000	cheesy, putrid
	Prot.	< 100	< 1,000	3,900,000		14,500,000	
41	Lip.	< 100	<1,000	400,000	sl. cheesy	3,000,000	cheesy
	Prot.	< 100	180,000	3,200,000		10,500,000	

xxx Too many to count

* unclean

by a marked increase in numbers of lipolytic and proteolytic bacteria. Rancidity was the most common flavor defect noted at the 28 day examination, while cheesy flavor was the most common defect in the unsalted butter after 56 days. In many cases the lots with rancid flavor at 28 days had developed a pronounced cheesy flavor at 56 days.

Comparison of the changes in numbers of bacteria and the appearance of flavor defects in unsalted butter at different temperatures

Table XL presents a summary of the changes in numbers of bacteria and of the occurrence of flavor defects in unsalted butter held at the different temperatures. The numbers of bacteria increased at each of the holding temperatures. A comparison of the trends in numbers of bacteria at the different temperatures indicates that corresponding high points were reached after longer holding periods with the lower temperatures. These points represent breaks in the trends of counts which were followed either by decreases or by much slower increases in counts. The corresponding values were noted as follows:

After 4 days holding at 21° to 26° C.

After 28 days holding at 5° C.

After 28 days holding at 0° C.

In general, the bacterial counts increased more rapidly at the higher holding temperatures. The numbers of bacteria increased most rapidly in the butter held at 21° to 26° C. but the numbers failed to reach as high a level within 7 days at this temperature as was reached after longer periods of holding at either 5° or 0° C. The highest count was recorded

Table XL

A Comparison of the Numbers of Total Bacteria and the Occurrence of Flavor Defects in Unsalted Butter Held at Different Temperatures

Numbers of bacteria expressed as geometric means

Temperature of holding		21° - 26° C.	5° C.	0° C.
Number of churnings		16	16	16
1st examination	Age of butter	0 days	0 days	0 days
	Bacteria per ml.	315,000	315,000	315,000
2nd examination	Age of butter	2 days	7 days	14 days
	Bacteria per ml.	26,350,000	470,800*	1,150,000
	Number defective lots	three	one	one
3rd examination	Age of butter	4 days	14 days	28 days
	Bacteria per ml.	38,660,000	9,150,000	35,160,000
	Number defective lots	seven	five	fourteen
4th examination	Age of butter	7 days	28 days	56 days
	Bacteria per ml.	35,510,000	50,840,000	71,710,000
	Number defective lots	ten	fourteen	sixteen
5th examination	Age of butter		56 days	
	Bacteria per ml.		51,180,000	
	Number defective lots		sixteen	

* G. M. of 15 lots

on the butter held 56 days at 0° C.

The more rapid increase in numbers of bacteria in unsalted butter at the higher temperatures suggests that bacteriological deterioration in this butter would be accelerated as the temperature of holding was increased. The relationship of the changes in counts to the occurrence of flavor defects is shown in Table XL.

The occurrence of defects in the butter agreed in general with the extensive increases in total bacterial counts on butter. The bacterial counts had increased extensively in the butter after 28 days at 5° and 0° C. and fourteen of the sixteen lots were defective at the 28 day examination. At room temperature the relationship between increase in counts and the occurrence of defective lots was not as close, since only seven of the sixteen lots became defective within the 4 day period even though the highest average count for this temperature was recorded at this time.

Comparison of numbers of lipolytic and proteolytic bacteria in unsalted butter held at different temperatures

The ranges of the numbers of lipolytic bacteria in the unsalted butter held at different temperatures are given in Table XLI. In general, the numbers of lipolytic bacteria increased at each of the holding temperatures but the rate and extent of the increases varied considerably. The increases at 5° C. were more extensive than at either 21° to 26° C. or at 0° C. The numbers of lipolytic bacteria exceeded 1,000,000 per ml. in 81.2 per cent of the lots held at 5° C. while 56.2 per cent of the lots held at 0° C. reached counts of over 1,000,000 per ml. The numbers of

Table XLI

Numbers of Lipolytic Bacteria in Unsalted Butter

Held at Different Temperatures

Temperature of holding	Time of plating	Number of lots	Range in numbers of lipolytic bacteria per ml.	Percentage of lots developing over 1,000,000 per ml.
	0 days	16	100 - 500	
21° - 26° C.	2 days 4 days 7 days	16	10,000 - 1,350,000 10,000 - 1,200,000 10,000 - 1,950,000	12.5
5° C.	7 days 14 days 28 days 56 days	16	100 - 1,800,000 1,000 - 6,000,000 10,000 - 20,000,000 10,000 - 30,000,000	81.2
0° C.	14 days 28 days 56 days	16	100 - 700,000 10,000 - 18,000,000 10,000 - 23,000,000	56.2

lipolytic bacteria exceeded 1,000,000 per ml. in only 12.5 per cent of the lots held at 21° to 26° C.

Table XLII presents the ranges of numbers of proteolytic bacteria in the unsalted butter held at different temperatures. The numbers of proteolytic bacteria increased extensively at each of the holding temperatures. The increases were more extensive at 5° and 0° C. than at 21° to 26° C., as indicated by the fact that at either 5° or 0° C. 87.5 per cent of the lots developed over 1,000,000 proteolytic bacteria per ml., while only 50 per cent of the corresponding lots held at 21° to 26° C. developed over 1,000,000 proteolytic bacteria per ml.

Changes in numbers of total bacteria in salted butter held at different temperatures

Table XLIII presents a comparison of the trends in total bacterial counts on salted butter held at different temperatures. A small increase in numbers of total bacteria occurred in the butter held at 21° to 26° C. while at the lower temperatures the numbers of bacteria decreased gradually to the end of the 56 day holding period.

No data on the numbers of lipolytic or proteolytic bacteria in salted butter are presented since the plates made with dilutions as low as 1:100 failed to show colonies of these types. Flavor defects other than tallowy were not noted in the salted butter during the periods of observation.

Comparative time required for flavor defects to develop in butter held at different temperatures

Table XLII

Numbers of Proteolytic Bacteria in Unsalted Butter Held at Different Temperatures

Temperature of holding	Time of plating	Number of lots	Range in numbers of proteolytic bacteria per ml.	Percentage of lots developing over 1,000,000 per ml.
	0 days	16	100 - 92,000	
21° - 26° C.	2 days 4 days 7 days	16	10,000 - 10,900,000 10,000 - 61,000,000 10,000 - 16,000,000	50
5° C.	7 days 14 days 28 days 56 days	16	100 - 2,650,000 1,000 - 16,500,000 10,000 - 17,000,000 10,000 - 30,000,000	87.5
0° C.	14 days 28 days 56 days	16	100 - 4,000,000 10,000 - 16,000,000 10,000 - 26,000,000	87.5

Table XLIII

Changes in Numbers of Total Bacteria in Salted Butter Held at Different Temperatures

Numbers of bacteria expressed as geometric means

Temperature of holding		21° - 26° C.	5° C.	0° C.
Number of churnings		16	16	16
1st examination	Age of butter	0 days	0 days	0 days
	Bacteria per ml.	115,000	115,000	115,000
2nd examination	Age of butter	2 days	7 days	14 days
	Bacteria per ml.	129,000	58,200*	60,100
3rd examination	Age of butter	4 days	14 days	28 days
	Bacteria per ml.	203,000	61,600	34,400
4th examination	Age of butter	7 days	28 days	56 days
	Bacteria per ml.	464,000	42,700	18,300
5th examination	Age of butter		56 days	
	Bacteria per ml.		19,100	

* G. M. of 15 churnings

The flavor defects appearing in the unsalted butter of churnings 26 to 41 have been reported in previous tables for comparison with the occurrence of lipolytic and proteolytic bacteria. Table XLIV gives a summary showing the comparative flavor deterioration in unsalted butter held at different temperatures.

Of the 14 churnings which showed flavor defects within 28 days at 5° C. only nine became defective in flavor within 7 days at 21° to 26° C. The two churnings which kept at 5° C. for 28 days failed to keep for 7 days at 21° to 26° C. There was some indication that the unsalted butter which deteriorated within 7 days when held at 21° to 26° C. showed flavor deterioration sooner when held at 5° C. than the churnings which kept at the higher temperature.

Of the thirteen churnings which showed flavor deterioration within 28 days at 0° C., only eight became defective in flavor within 7 days at 21° to 26° C. The three churnings which kept at 0° C. for 28 days failed to keep for 7 days at 21° to 26° C. There was no apparent difference in keeping quality at 0° C. between the churnings which kept and those which failed to keep for 7 days at 21° to 26° C.

The results in Table XLIV show that, in certain cases, flavor defects did not appear in unsalted butter held at 21° to 26° C. when flavor deterioration occurred in the corresponding butter at lower temperatures. The mild and indefinite character of the flavor defects in certain lots held at 21° to 26° C. suggested that additional time might bring out defects which were probably being overlooked. The results in Table XLV include observations on a new series of eighteen churnings of unsalted butter made

Table XLIV

Comparison of Time of Appearance and Nature of Flavor Defects in Unsalted Butter Held at Different Temperatures

Churning no.	Flavor comments on butter held at					
	21° - 26° C.		5° C.		0° C.	
	Days	Flavor defect	Days	Flavor defect	Days	Flavor defect
26	4	roquefort	28	sl. rancid	28	sl. rancid
	7	roquefort	56	rancid, fruity	56	rancid, cheesy
27	4	roquefort	14	sl. unclean	28	sl. cheesy
	7	roquefort	28	cheesy, rancid	56	cheesy
28			28	sl. rancid	28	sl. rancid
	7	---	56	sl. cheesy	56	sl. rancid
29	7	sl. fermented	56	sl. rancid	56	sl. cheesy
30			14	sl. rancid	28	sl. rancid
	7	rancid	28	fruity, rancid	56	fruity, cheesy
31	2	sour	28	unclean	28	sl. off
	7	cheesy	56	roquefort, rancid	56	sl. rancid
32	7	rancid	56	cheesy, putrid	56	fruity, cheesy
33			28	fruity, rancid	28	sl. rancid
	7	sl. cheesy	56	cheesy, putrid	56	cheesy
34	2	sour	28	sl. rancid		
	4	roquefort	56	rancid, cheesy	56	sl. cheesy
35	2	sl. sour	28	fruity, rancid	28	fruity, rancid
	4	sl. rancid	56	cheesy	56	cheesy
36			28	fruity, rancid	28	sl. rancid
	7	---	56	cheesy	56	cheesy
37			28	cheesy	28	sl. off
	7	---	56	fruity, rancid	56	fruity, rancid
38			28	sl. cheesy	28	sl. cheesy
	7	---	56	cheesy	56	cheesy
39	4	sour	7	cheesy	14	unclean
	7	cheesy	14	cheesy	28	sl. rancid
40			14	sl. cheesy	28	sl. off
	7	---	28	sl.cheesy,unclean	56	cheesy, putrid
41	4	sour	14	rancid	28	sl. cheesy
	7	sour	28	cheesy	56	cheesy

--- Flavor satisfactory

Table XLV

Comparison of Time of Appearance and Nature of Flavor Defects in Unsalted Butter Held at Different Temperatures

Churning no.	Flavor comments on butter held at					
	21° - 26° C.		5° C.		0° C.	
	Days	Flavor defect	Days	Flavor defect	Days	Flavor defect
42	7	sour	14	sl. off	14	sl. off
	10	sour	21	sl. cheesy	28	fruity, rancid
43	4	cheesy	7	sl. cheesy	21	rancid, cheesy
			21	putrid		
44	4	sl. cheesy	10	sl. cheesy	14	sl. cheesy
	7	cheesy	14	putrid	28	cheesy, unclean
45	4	rancid	21	fruity	21	sl. cheesy
	7	cheesy	28	cheesy	28	cheesy, rancid
46	7	sl. moldy	14	sl. cheesy	14	sl. unclean
	10	sl. cheesy	28	fruity	28	woody, oily
47	10	---	14	sl. cheesy	21	sl. off
			28	rancid	28	oily, putrid
48	4	sour	10	sl. cheesy	14	fruity
	7	cheesy	21	cheesy, rancid	28	rancid
49	10	---	14	fruity, rancid	14	sl. unclean
			28	cheesy, rancid	28	cheesy
50	10	---	56	---	56	---
51	10	sl. sour	14	sl. off	28	sl. off
			28	sl. rancid	56	cheesy
52	10	doughy	42	sl. cheesy	56	fruity
53	10	---	21	sl. rancid	21	sl. fruity
			28	sl. fruity	56	rancid, cheesy
54	10	---	56	---	56	---
55	10	---	21	flat	21	unclean
			28	cheesy	56	sl. stale
56	10	sl. sour	28	sl. fruity	28	sl. fruity
			56	fruity	56	cheesy
57	10	---	28	fruity	28	sl. fruity
			56	fruity	56	cheesy
58	7	cheesy	7	sl. alkaline	21	sl. fruity
			21	sl. cheesy	28	alkaline, putrid
59	7	sl. sour				
	10	rancid	56	cheesy	56	sl. fruity

--- Flavor satisfactory

without butter culture. Flavor examinations were made at the following periods:

4, 7, and 10 days at 21° to 26° C.

7, 10, 14, 21, 28, 42, and 56 days at 5° C.

7, 14, 21, 28, 42, and 56 days at 0° C.

The data include the first flavor detected and the final flavor defect noted on each lot. Pronounced flavor deterioration was evident within 28 days in the unsalted butter at either 5° or 0° C. In general, the flavor defects were more pronounced and appeared sooner at 5° C. than at 0° C. Of the sixteen lots which deteriorated at both 5° and 0° C., eleven showed flavor defects within 10 days at 21° to 26° C. In two churnings flavor deterioration did not occur at any of the temperatures used. Flavor deterioration in unsalted butter within 10 days at 21° to 26° C. indicated poor keeping quality in butter held at 5° and 0° C. but failure to show deterioration at 21° to 26° C. did not insure good keeping quality at the lower temperatures in every case.

SUMMARY AND CONCLUSIONS

SECTION A

Part I

Butter Made With Butter Culture

1. When unsalted butter and serum from this butter were held at 21° C., a much more rapid and extensive flavor deterioration and a more rapid increase in numbers of bacteria occurred in the serum than in the corresponding butter.
2. The presence of salt in butter held at 21° C. effectively prevented flavor deterioration and reduced the numbers of bacteria, while the salt in serum restrained the growth of bacteria at 21° C. for only the first 2 to 4 days of the 7 day holding period and flavor deterioration occurred in four of the nine lots.
3. Lipolytic and proteolytic bacteria increased extensively in both the unsalted butter and the butter serum from certain churnings. No close correlation between the numbers of these types and specific flavor defects was noted in unsalted butter but in butter serum large numbers of proteolytic bacteria occurred concurrently with cheesy flavors. Proteolytic bacteria increased much more extensively in unsalted butter serum than in the corresponding butter.
4. The flavor defects occurring in unsalted, butter serum frequently suggested proteolytic decomposition, while the flavor defects in un-

salted butter commonly suggested lipolytic changes.

5. Neither lipolytic nor proteolytic bacteria were found in the salted butter or butter serum held at 21° C.
6. A study of flavor deterioration in unsalted butter serum at 21° C. did not aid materially in the prediction of the keeping quality of the corresponding butter held at 21° C. The flavor defects appeared sooner in the serum than in the corresponding butter but they were frequently quite different from those produced in the butter.

Part II

Butter Made With Butter Culture

1. Freezing at -25° C. effected marked decreases in the numbers of total bacteria present in either salted or unsalted butter but the decreases were much less marked in the salted butter than in the corresponding unsalted butter. The destructive action of salt was apparently of greater importance than freezing in reducing the numbers of bacteria in salted butter.
2. Freezing reduced the numbers of lipolytic and proteolytic bacteria in salted and unsalted butter as indicated by the absence of these types on the plates made from the frozen butter. Lipolytic and proteolytic bacteria developed in a majority of the unsalted lots held at 21° C. subsequent to freezing, indicating that bacteria of these types were not completely destroyed.
3. The numbers of total bacteria increased more rapidly in unsalted butter held at 21° C. subsequent to freezing than in the fresh butter

held under the same conditions.

4. Flavor deterioration was more rapid and extensive in unsalted butter held 7 days at 21° C. subsequent to freezing than in the fresh butter held under the same conditions.
5. The development of flavor defects in unsalted butter held 7 days at 21° C. subsequent to freezing was often accompanied by large increases in numbers of lipolytic and proteolytic bacteria.
6. The numbers of total bacteria did not change significantly in salted butter held 7 days at 21° C. subsequent to freezing.
7. Neither lipolytic nor proteolytic bacteria were detected in the salted butter which had been held 7 days at 21° C. subsequent to freezing.
8. Flavor defects other than tallowiness were not detected in the salted butter when it was held 7 days at 21° C. subsequent to freezing.

Part III

Butter Made With Butter Culture

1. The numbers of total bacteria generally increased in unsalted butter held at 21°, 15°, 5°, or 0° C. The rates of increase were greater with the higher temperatures. Counts of similar magnitude were reached after about 2 days at 21° C., 7 days at 15° C., and 56 days at 0° C.
2. The numbers of total bacteria in salted butter generally decreased at 21°, 15°, 5°, or 0° C. The rates of decrease were greater with the higher temperatures. The counts were reduced to similar levels after about 4 days at 21° C., 7 days at 15° C., and 56 days at 0° C.
3. The numbers of total bacteria, as indicated by the G. M. of counts be-

fore and after holding at each temperature, increased in the unsalted butter from twenty to fiftyfold while on the corresponding salted butter the numbers after holding were from two-fifths to one-sixth of the initial numbers.

4. Lipolytic and proteolytic bacteria were either very few or were not detected in the fresh, unsalted butter and were regularly not detected in the fresh, salted butter.
5. The numbers of lipolytic and proteolytic bacteria generally increased in the unsalted butter held at 21° C. but the presence of these types was not closely correlated with the development of either rancid or cheesy flavors in individual lots. Only three lots became rancid and cheesy flavors were not detected in any of the lots held 7 days at 21°C.
6. The numbers of lipolytic and proteolytic bacteria increased extensively in unsalted butter held at either 15° or 5° C. There appeared to be a close correlation between the appearance of large numbers of lipolytic bacteria and the development of typical rancidity at either temperature. Flavor defects denoting proteolysis were not frequently detected even though large numbers of proteolytic bacteria were commonly present in unsalted butter after holding at either 15° or 5° C.
7. The numbers of lipolytic and proteolytic bacteria increased in fourteen of the fifteen lots of unsalted butter held at 0° C. In general, the lots which developed rancidity contained large numbers of lipolytic bacteria; however, large numbers of lipolytic bacteria were not always accompanied by rancidity. A flavor denoting proteolysis was detected in only one lot even though proteolytic bacteria were found frequently in the unsalted butter after holding at 0° C.

8. Lipolytic bacteria occurred more commonly and counts of over 1,000,000 per ml. more frequently in unsalted butter held at 15° or 5° C. than in the corresponding lots held at either 21° or 0° C. Proteolytic bacteria occurred more commonly and counts of over 1,000,000 per ml. were reached more frequently at 5° C. than at any of the other temperatures studied.

9. There appeared to be a general agreement between the time of appearance of flavor defects and the time at which the highest level of total bacterial counts had been reached in the unsalted butter at each of the temperatures except 21° C.

10. Flavor deterioration in unsalted butter within either 7 or 10 days at 21° C. indicated flavor deterioration within 56 days at either 5° or 0° C. but a failure to show flavor deterioration at 21° C. did not insure good keeping quality at the lower temperatures.

11. The flavor deterioration at 15° C. compared more closely with flavor deterioration at lower temperatures than did the results at 21° C. but the holding test at 15° C. required a longer time before flavor deterioration became apparent.

12. Neither lipolytic nor proteolytic bacteria were detected in the salted butter after holding at any of the temperatures.

13. Flavor deterioration other than tallowiness was not detected in salted butter after holding at any of the temperatures.

SECTION B

Butter Made Without Butter Culture

1. The numbers of total bacteria generally increased in unsalted butter held at temperatures of 21° to 26°, 5° or 0° C., The rates of increase were greater with the higher temperatures. Counts of similar magnitude were obtained after about 4 days at 21° to 26° C., 28 days at 5° C., and 28 days at 0° C.
2. The numbers of total bacteria increased slightly in salted butter held at 21° to 26° C. while at 5° or 0° C. marked decreases in the numbers occurred.
3. The numbers of total bacteria, as indicated by the G. M. of counts before and after holding at each temperature, increased in the unsalted butter from one hundred to two hundredfold while the numbers in salted butter at 5° to 0° C. decreased to one-sixth of the initial numbers, and at 21° to 26° C. increased about fourfold.
4. Lipolytic bacteria were usually not detected in fresh unsalted butter. Proteolytic bacteria were found in about half of the lots of fresh unsalted butter and in those lots which yielded proteolytic bacteria, the numbers ranged from 200 to 25,000 per ml.
5. Lipolytic bacteria were not frequently detected in unsalted butter held at 21° to 26° C. Proteolytic bacteria increased rather extensively and although the lot containing the largest number of proteolytic bacteria developed a cheesy flavor, there was usually no correlation between the

numbers of proteolytic bacteria and flavor deterioration.

6. Large numbers of lipolytic and proteolytic bacteria were commonly associated with the development of flavor defects in unsalted butter held at either 5° or 0° C.

7. The time at which the largest number of defective lots of unsalted butter were noted at each temperature agreed, in general, with the time at which the average bacterial contents reached the highest level. This relationship was only a general one, however, as indicated by the fact that no close correlation could be noted between the numbers of total bacteria in individual lots and the development of flavor defects.

8. Flavor deterioration in unsalted butter within 7 or 10 days at 21° to 26° C. frequently indicated flavor deterioration in the corresponding butter held at lower temperatures, but a failure to show flavor deterioration at 21° to 26° C. did not insure good keeping quality in the butter at lower temperatures.

9. Neither lipolytic nor proteolytic bacteria were noted in salted butter held at any of the temperatures.

10. Flavor deterioration other than tallowiness was not detected in salted butter held at any of the temperatures.

GENERAL CONCLUSIONS

1. A "holding test", consisting of holding small portions of unsalted butter for 7 to 10 days at 21° C., gives useful information on the keeping quality of unsalted butter at lower temperatures.
2. The salted butter included in this investigation did not show flavor deterioration, except for tallowiness at the higher temperatures of holding, therefore no information was obtained on the value of a"holding test" for the prediction of keeping quality of salted butter.
3. The increases in numbers of total bacteria and the flavor deterioration in unsalted, non-culture butter were much more extensive than in the unsalted, culture butter held under similar temperature conditions.
4. The growth of bacteria in unsalted butter at 21° C. was apparently not as much of a factor in flavor deterioration as the growth of similar numbers of bacteria in unsalted butter at lower temperatures. The more extensive development of lipolytic and proteolytic bacteria at the lower temperatures than at 21° C. was indicated as the reason for this condition.
5. The bacterial colonies which showed lipolytic activity on plates made from defective butter,in many cases, also showed proteolytic activity. The flavor defects in unsalted butter suggested that fat splitting and casein digestion often occurred in the same sample.
6. The development of rancidity in unsalted butter at either 5° or 0° C. was frequently accompanied by large numbers of lipolytic bacteria and cheesy flavors were often accompanied by large numbers of proteolytic

bacteria under the same holding conditions.

7. Salt in butter at the rate of 2.5 per cent effectively prevented the growth of lipolytic and proteolytic bacteria under the holding conditions. of this investigation.

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