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A Holding Test at Room Temperature as an Indication of Keeping Quality of Butter in Storage

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A Holding Test at Room Temperature as an Indication of the Keeping Quality of Butter in Storage

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Summary

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Butter Made With Butter Culture

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2. The presence of salt in butter held at 21 degrees C., effectively prevented flavor deterioration and reduced the numbers of bacteria, while the salt in serum restrained the growth of bacteria 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 unsalted butter and 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 the serum large numbers of proteolytic bacteria occurred concurrently with cheesy flavors.

4. Neither lipolytic nor proteolytic bacteria were found in the salted butter or butter serum held at 21 degrees C.

5. A study of flavor deterioration in unsalted butter serum at 21 degrees C. did not aid materially in the prediction of the keeping quality of corresponding butter held at 21 degrees 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. The numbers of total bacteria generally increased in unsalted butter held at 21, 15, 5, or 0 degrees C. The rates of increase were greater with the higher temperatures. Counts of similar magnitude were reached after about two days at 21 degrees C., seven days at 15 degrees C., 21 days at 5 degrees C., and 56 days at 0 degrees C.

2. The numbers of total bacteria in salted butter generally decreased at 21, 15, 5, or 0 degrees C. The rates of decrease were greater with the higher temperatures. The counts were reduced to similar levels after about four days at 21 degrees C., seven days at 15 degrees C., 21 days at 5 degrees C., and 56 days at 0 degrees C.

3. The numbers of lipolytic and proteolytic bacteria generally increased in the unsalted butter held at 21, 15, 5 and 0 degrees C. The presence of these types in butter at 21 degrees C. was not closely correlated with the development of either rancid or cheesy flavors in individual lots. At either 15 , 5, or 0 degrees C., there appeared to be a close correlation between the appearance of large numbers of lipolytic bacteria and the development of typical rancidity. Flavor defects denoting proteolysis were not frequently detected even though large numbers of proteolytic bacteria were commonly present in unsalted butter after holding at these temperatures.

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

5. 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 unsalter butter at each of the temperatures except 21 degrees C.

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

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

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

9. Flavor deterioration other than tallowness 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 21-26, 5 or 0 degrees C. The rates of increase were greater with the higher temperatures. Counts of similar magnitude were obtained after about four days at 21-26 degrees C., 28 days at 5 degrees C., and 28 days at 0 degrees C.

2. The numbers of total bacteria increased slightly in salted butter held at 21-26 degrees C. while at 5 or 0 degrees C. marked decreases in the numbers occurred.

3. Lipolytic bacteria were usually not detected in fresh unsalted butter and proteolytic bacteria were found in only about one half of the lots of fresh unsalted butter.

4. 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 degrees C.

5. 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.

6. Flavor deterioration in unsalted butter within seven or ten days at room temperature frequently indicated flavor deterioration in the corresponding butter held at lower temperatures, but a failure to show flavor deterioration at room temperature did not insure good keeping quality in the butter at lower temperatures.

7. Neither lipolytic not proteolytic bacteria were noted in salted butter held at any of the temperatures.

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

A Holding Test Made at Room Temperature as an Indication of the Keeping Quality of Butter in Storage[1]

by D. H. Jacobsen[2]

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 two weeks to two 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

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[2]The author wishes to express his sincere appreciation to Dr. B. W. Hammer for counsel and assistance in planning and carrying out this work, and to Prof. M. Mortensen and other members of the Department of Dairy Industry of Iowa State College where a part of this work was done.

the flavor deterioration over a period of seven 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 degrees 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 degrees 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 degrees C. were compared with the rate of deterioration and the flavor defects occuring at lower temperatures. Such comparisons were used in judging the reliability of the changes taking place in butter at 21 degrees 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 (22) 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 (12) in 1906, studied the effect of different storage temperatures on butter quality and found that —10 degrees F. was superior to any of the higher temperatures tried. When stored at this temper-

ature 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 (35) compared the loss in score on butter stored at 0, 10 and 20 degrees F. The study included raw cream butter and pasteurized ripened cream butter and pasteurized unripened cream butter. Storage at 0 degrees F. gave the best results. The advantage of 0 degrees F. over 10 degrees 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 (37) concluded that bacteria might cause butter deterioration without any multiplication. Samples of salted butter held at 21 degrees F. showed slowly increasing bacterial counts but no definite relationship between this increase and flavor deterioration was detected.

Rogers, Berg, Potteiger, and Davis (34) investigated the factors which influence the change in flavor of storage butter. Experiments with raw, pasteurized, and pasteurized cream reinoculated with cultures from the 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 (44) compared the changes in bacterial counts and in score of salted and unsalted butter held for 284 days at 15 degrees F. followed by 20 days at 58 degrees F. The bacteria in unsalted butter decreased more rapidly at 15 degrees F. than they did in salted butter and increased more rapidly at 58 degrees F. 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 (4) studied the types and numbers of microorganisms occurring in salted butter at the end of various periods of storage up to one year at 32 degrees F. 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 (32) 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 (13) considered the action of the bacteria, yeasts and molds in butter stored at 6 degrees F. 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 (36) 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.

Demeter and Maier (9) compared the score and microbiological composition of 500 samples of pastuerized sour cream butter which were stored

at about 38 degrees F. for a period of 10 days. No specific relationship was found between the microflora of the butter and such flavors as rancid, cowy, oily, and unclean.

Grimes (14) 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 degrees C. for a period of two weeks. No definite correlations were noted between the flavor score and either the microbiological content or acidity of the butter. Wide variations in numbers of liquefying bacteria, yeasts and molds occurred in each grade of butter after the two weeks storage.

Loftus-Hills, Scharp, and Bellair (25) considered the factors influencing the keeping qualities of Victorian, salted butter stored at 12 degrees F. for three 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 (15) reported similar results when they stored sweet cream, salted butter at 15 degrees F. for two to eight months.

Nelson (28) observed the changes in numbers of bacteria in butter held for seven days at 21 degrees C. by means of the microscopic method developed by Hammer and Nelson (20) 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.

Shepard (38) held salted and unsalted butter at 0 and 21 degrees 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 degrees 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 degrees 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 (17) 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 degrees 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.

Although total bacterial counts have not been definitely correlated with flavor deterioration in butter the relationship of bacteria to certain specific flavor defects has been recognized. The specific flavor defects which have been considered to be of microbial origin are rancid, cheesy, and unclean.

The exact nature of butter flavor defects referred to in the literature as rancid is somewhat uncertain. Some early investigators, no doubt, referred to all butter which has turned bitter or strong as rancid. In this

review only those investigations which deal with hydrolytic rancidity are reported. Guthrie (16) 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.

Reinmann (33) 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.

Orla Jensen (30) isolated a number of types of organisms which were associated with rancidity in butter. Some of the most common types found were **Oidum lactis, Cladosporium butyri,** Mycoderma varieties, lactose fermenting yeasts, and **Penicillium glaucum.** His work indicated that these microorganisms and the oxygen of the air were the chief causes of rancidity. Investigations by Stokoe (41) into the cause of rancidity in butter and oleomargarine indicated that this defect in both products was caused by microorganisms. Gratz (11) concluded that microorganisms were of prime importance in the development of rancidity in butter. He found that the numbers of lipolytic microorganisms and the activities of their enzymes determined the fat splitting. **Odium lactis** was found to be one of the most active agents in the development of rancidity.

Collins (5) studied the changes in numbers of lipolytic bacteria in unsalted raw cream butter at 6 degrees 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 (6) found that unsalted pasteurized cream butter made in a carelessly cleaned churn very frequently developed rancidity when held at 0 degrees C. for seven 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 (24) 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 degrees C.

Olson and Hammer (29) in a study of the keeping quality 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 and 45 degrees F. and scored at various intervals until definite flavor defects developed. Rancidity developed much more quickly in the samples held at 45 degrees F. than in those held at 32 degrees F.

Hammer and Collins (18) estimated 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 degrees C. the counts varied from less than 1,000 to 40,000 per ml. In the 10 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 seven 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 Colline and Hammer (7) 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 degrees C. Of these 80 cultures, 60 (75.0 per cent) produced rancidity in the butter.

Shepard (38) used the Nile-sulphate subject method to detect the numbers of lipolytic bacteria in butter held at 0 degrees C. Marked deterioration of the unsalted butter occurred at 0 degrees 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 (31) 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 degrees F. no correlation between the numbers of microorganisms and flavor deterioration could be noted. Brown (3) 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 (23) reported that protein decomposition was greater in raw cream butter than in pasteurized cream butter. They concluded that proteolysis was accelerated by microogranisms 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.

Spitzer, Parfitt, Manhart, and Epple (40) 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 (39) showed that bacterial cultures inoculated into butter tended to increase proteolysis. The numbers of total and gelatine liquefying bacteria were determined on 69 samples of contest butter held for 3 months at 0 to 4 degrees 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.

Among the butter defects caused by enzyme action, Virtanen (43) listed fermented, boiled, cheese-sour, putrified, and rank. He indicated that, as a rule, the enzymes causing these defects were produced by gelatine liquifying water bacteria, although yeasts and molds were also possible sources.

Indications that cheesiness is a common defect of unsalted butter are presented in the report of Derby and Hammer (10). By inoculating cream with cultures of bacteria isolated from surface-taint butter they found

that cheesy flavors associated with protein decomposition were produced. Nelson (28) noted that the most common defects encountered in butter samples held at 21 degrees C. for seven days were protein decomposition, cheesiness and putrid. Microscopic examinations made before and after holding indicated that bacteriological rather than chemical deterioration was responsible for the defects developed. Olson and Hammer (29) observed that unsalted butter from clean churns very frequently became cheesy after storage at either 32 or 45 degrees F.

Herreid, Macy, and Combs (21), 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 degrees C. or lower.

The importance of microorganisms in the development of butter flavors, other than rancidity and cheesiness, has not been well established. Cusick (8) 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 (42) 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 (2) suggested a keeping quality test consisting of observing the changes in small samples of butter held at 60 to 71 degrees F. for seven days. Their results showed that butter of poor keeping quality developed a bad flavor in three days while butter of good keeping quality had a satisfactory flavor after as long as two weeks.

Macy and Richie (26) 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 (27) held samples of butter at 37 degrees 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 catal-

ase and reductase content and developed off-flavors at 37 degrees C. were found to have poor keeping quality at lower temperatures.

Nelson (28) employed the microscopic examination of stained butter serum and a "holding test" consisting of holding small samples for seven days at 21 degrees 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 numberous 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 developed 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 degrees C. for 30 minutes. Churning was carried out in commercial churns of 100 to 750 pounds 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 five-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 degrees C. The 21 degrees and 15 degrees C. holding cabinets were thermostatically controlled to within ± 1 degree C. of the required temperatures. The 5, 0, and –25 degrees C. storage rooms were equipped with brine refrigeration coils and the temperature 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 degrees), 5 and 0 degrees C. The 5 degrees C. holding chamber was thermostatically controlled to within ± 1 degrees C. while the 0 degrees C. storage room was a regular holding room equipped with brine refrigeration coils.

Sampling Methods

Sterile wooden spatulas were used for removing all samples for plating and flavor inspection. 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 apearance of off-flavors in a sample.

The butter samples were plated when fresh and after the following holding periods:

21 degrees C. holding after 2, 4, and 7 days.
15 degrees C. holding after 7, 14, 21, and 28 days.
5 degrees C. holding after 7, 14, 21, 28, and 56 days.
0 degrees C. holding after 14, 28, and 56 days.
–25 degrees C. holding after 1 and 90 days.

Bacteriological Methods

The methods used in all plating procedures were adaptations of those described by the Committee on The Microbiological Analysis of Butter (1). The small portions of butter for plating included both surface and sub-surface material and were obtained by the use of sterile wooden spatulas.

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 room temperature 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.

The plates for total counts, as well as those for lipolytic and proteolytic counts, were incubated for three to four days at room temperature. 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 a 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.

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.

Experimental Results and Discussion

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. Ten of the fifteen churnings were made from sweet cream while five 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 degrees C. until churned. Section A includes 15 churnings from which both salted and unsalted butter were obtained for the study of bacteriological condition and flavor deterioration. Ten additional churnings of unsalted butter were studied for flavor deterioration only.

Part I

Comparison of Changes in Bacterial Content and of Flavor Deterioration in Butter and in Butter Serum Held at 21 Degrees 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 (19), 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 seven days at room

temperature (21 degrees 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 five-ounce portion in a water bath held at 40 to 45 degrees 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 degrees C. After two, four and seven days the samples were again plated and were also examined for flavor and odor.

A summary of the changes occurring in the numbers of bacteria in butter and butter serum is presented in Table I. It is evident from these results that a very different series of changes occured in the butter and in the serum separated from it. The bacterial counts on unsalted butter increased during the first two days but decreased slightly during the remainder of the seven-day period while bacterial counts on the serum showed a significant increase throughout the seven-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 two days and then decreased more slowly to the end of the seven days while the bacteria in salted butter serum decreased during the first two days but then increased sharply to the end of seven days.

TABLE I

Changes in Bacterial Counts on Butter and on the Corresponding Butter Serum Held 7 days at 21° C.

Days held	Number 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	58,300	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

The above results agree, in general, with those of Hammer and Hussong (19) for the holding period which they investigated. Their results covering the changes in bacterial counts during two 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 I, however, occurred largely after the two-day holding period. The bacterial counts also range 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 trials 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 Degrees C.—A comparison of the flavor defects appearing in butter and in the corresponding serum held seven days at 21 degrees C. is presented in Table II. 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 seven-day period.

A comparison of the flavor deterioration in butter and serum recorded in Table II and the changes in bacterial counts on the corresponding butter show some interesting relationships. The rapid flavor deterioration in the unsalted serum was accompanied by a marked increase 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 but the corresponding lots of salted butter kept for the seven-day period.

TABLE II

A Comparison of the Development of Flavor Defects in Butter and in the Corresponding Butter Serum held 7 days at 21° C.

Churning	Unsalted						Salted			
	Butter		Serum				Butter		Serum	
no.	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	metalic	7	------	7	bitter
9	7	------	4	fermented	7	cheesy	7	------	7	bitter

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

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 and Proteolytic Bacteria to the Development of Flavor Defects in Unsalted Butter and in the Corresponding Serum at 21 Degrees 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 III presents a comparison of the numbers of lipolytic bacteria in unsalted butter and butter serum with the flavor defects noted during seven days at 21 degrees 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 from less than 10,000 to 23,000,000 per ml.

TABLE III

Numbers of Lipolytic Bacteria and Flavor Defects in Unsalted Butter and in Corresponding Butter Serum at 21° C.

Churning no.		0 Days lipolytic bacteria per ml.	2 Days Defect	2 Days lipolytic bacteria per ml.	4 Days Defect	4 Days lipolytic bacteria per ml.	7 Days Defect	7 Days 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	fermented	< 1,000		< 10,000
	Serum	10,000		< 1,000		< 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	metalic	< 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

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.

Some of the flavor defects which developed in the unsalted butter and butter serum appeared to be the result of proteolysis rather than fat splitting. Table IV gives the numbers of proteolytic bacteria and the flavor defects in the unsalted butter and butter serum held seven days at 21 degrees C. The numbers of proteolytic bacteria were usually much greater in unsalted serum than in the corersponding 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 seven days while all of the lots of serum deteriorated within 4 days.

TABLE IV

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

Churning no.		0 Days proteolytic bacteria per ml.	2 Days Defect	2 Days proteolytic bacteria per ml.	4 Days Defect	4 Days proteolytic bacteria per ml.	7 Days Defect	7 Days 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		< 1,000	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		< 1,000	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

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 proteolytic 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 degrees 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 butter in the corresponding serum.—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 preventd the development of the off-flavors usually attributed to them.

Part II

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 15 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 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 Total Bacteria in Salted and Unsalted Butter Held at Different Temperatures—A comparison is presented in Fig. 1 of the trends in numbers of bacteria in unsalted butter at different temperatures. Corresponding high points were reached after a longer holding period with each successively lower temperature. These high points do not necessarily represent the maximum numbers reached at the different temperatures but rather the points at which 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 degrees C.
After 7 days holding at 15 degrees C.
After 21 days holding at 5 degrees C.
After 56 days holding at 0 degrees 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 degrees C., but the counts failed to reach as high a level within seven days at 21 degrees C., as was reached in the butter held for longer periods at the lower temperatures, viz. 15, 5 and 0 degrees 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.

A comparison of the changes in numbers of total becteria in salted butter, Fig. 1 indicates that the numbers decrease more rapidly as the temperature increased. The corresponding low points in counts coincided in general with the time at which high points were noted in the unsalted butter.

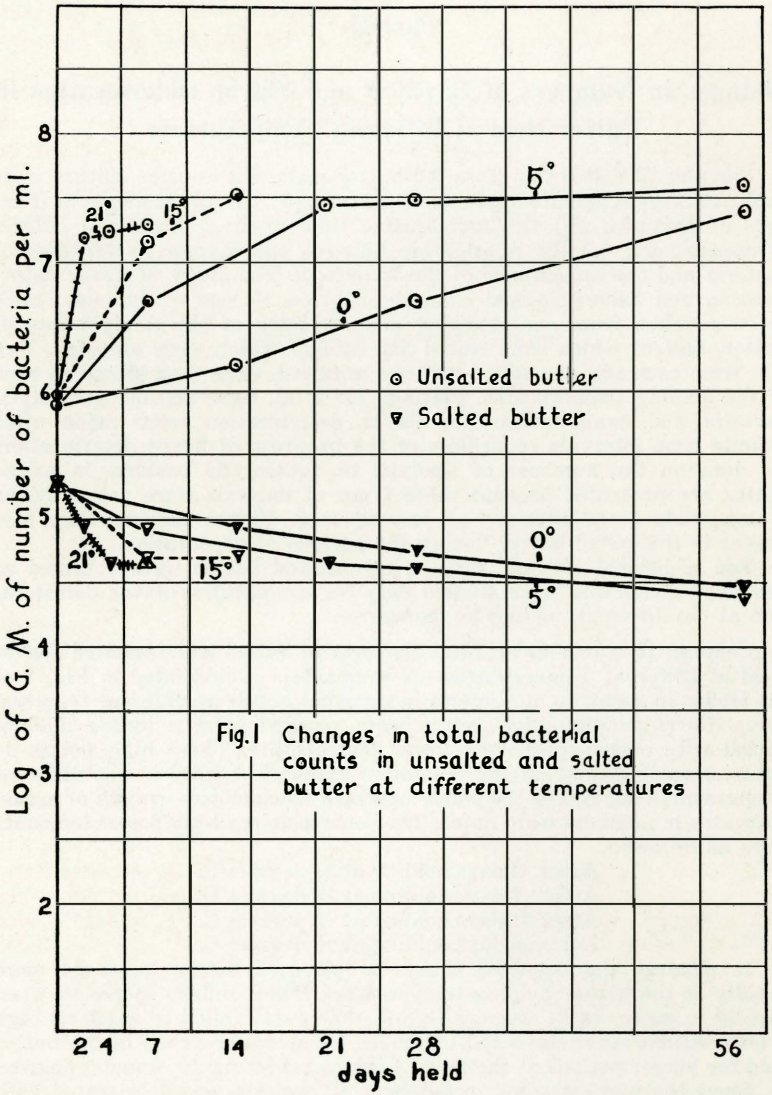

Fig. 1 Changes in total bacterial counts in unsalted and salted butter at different temperatures

Temperatures recorded in chart should be read degrees C.

Comparison of the Changes in Numbers of Bacteria and the Occurrence of Flavor Defects in Unsalted Butter Held at Different Temperatures—The preceding comparison showed that the numbers of bacteria in unsalted butter increased more rapidly as the holding temperatures increased. The relationship of such bacterial activity to flavor deterioration in unsalted butter is indicated in Table V by a comparison of the trends in counts and the appearance of flavor defects.

TABLE V

A Comparison of the Numbers of Total Bacteria and the Occurence of Flavor Defects in Unsalted Butter Held at Different Temperatures
Numbers of bacteria expressed as geometric means

Temp. of holding		21° C.	15° C.	5° C.	0° C.
Number of cuhrnings		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	

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 degrees C. At this temperature, only three of the 15 lots were defective at four days in spite of the fact that the counts were comparatively high at this time.

All of the lots held at 15 degrees C. were defective in flavor within 14 days and the highest count at this temperature was also noted at this time. Twelve of the 15 lots held at 5 degrees C. were defective in flavor at 21 days and at this time the counts had reached a level corresponding to the highest counts at 21 degrees and 15 degrees C. The greatest number of defective lots in the butter at 0 degrees C. were found at the 56-day examination 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 degrees C. suggests that a comparison of the types of bacteria at these different temperatures might offer some explanation. The temperature of 21 degrees 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—A summary showing the numbers of lipolytic bacteria in unsalted butter held at different temperatures is given in Table VI. The numbers of lipolytic bacteria in

TABLE VI

Numbers of Lipolytic Bacteria in Unsalted Butterfat Held at Different Temperatures

Temperature of holding	Time of Plating	Number of lots	Range in numbers of lypolytic 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

the fresh butter were very small as is indicated by the range in counts from less than 100 to 3,600 per ml. The numbers of lipolytic bacteria increased in unsalted butter at all of the different temperatures of holding as indicated by the range of counts and by the percentage of the lots in which the numbers of lipolytic bacteria exceeded 1,000,000 per ml. at sometime during holding. The numbers of lipolytic bacteria increased more regularly and reached above 1,000,000 per ml. more frequently at 15 and 5 degrees C. than at 21 and 0 degrees C. All of the lots held at 15 degrees 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 degrees C. all lots showed lipolytic bacteria at the 28th 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 and 0 degrees C. was much more irregular. In either case the numbers reached 1,000,000 in only 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 VII. 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 degrees 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 degrees C. showed proteolytic bacteria at the 14 and 28 day examinations, and 71.4 per cent of the lots developed

TABLE VII

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	15	< 100 — 2,500,000	40
	4 days		< 1,000 — 2,750,000	
	7 days		< 10,000 — 3,500,000	
15°C.	7 days	7	< 1,000 — 1,100,000	71.4
	14 days		500,000 — 1,500,000	
	28 days		15,000 — 8,500,000	
5°C.	7 days	15	< 100 — 1,900,000	93.3
	21 days		< 10,000 — 18,000,000	
	28 days		< 10,000 — 23,000,000	
	56 days		< 10,000 — 16,000,000	
0°C.	14 days	15	< 100 — 590,000	73.3
	28 days		< 1,000 — 4,900,000	
	56 days		< 10,000 — 15,500,000	

more than 1,000,000 per ml. The numbers of proteolytic bacteria increased to more than 1,000,000 per ml. in 73.3 per cent of the lots at 0 degrees C., and in 40 per cent of the lots held at 21 degrees 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 degrees C. than in butter at either higher or lower temperatures.

Time of Appearance and Nature of Flavor Defects in Unsalted Butter Held at Different Temperatures—Table VIII presents a summary of the defects occurring on each sample and includes the defect first noted and the final defect developed during holding. The periods at which flavor examinations were made were the same as those used in making the bacteriological examinations reported in previous tables.

For the purpose of showing the value of a holding test at room temperature for the prediction of keeping qualities of butter at the lower temperatures, the following comparisons are presented. Seven lots of unsalted butter held at 15 degrees C. showed flavor defects after 14 days and only three of these developed flavor defects within seven days at 21 degrees C. Fifteen lots of unsalted butter held at 5 degrees C. showed flavor defects after periods varying from 21 to 28 days and only nine of these lots showed flavor defects during seven days at 21 degrees C. Eleven lots of unsalted butter held at 0 degrees C. showed flavor defects within the 56 day period and only seven of these lots showed flavor defects during seven days at 21 degrees C. The off-flavors which were noted in unsalted butter at 21 degrees C. were generally of a milder and less definite nature than those noted at lower temperatures. Furthermore, the growth of butter culture organisms in the butter at 21 degrees C. resulted in a definite ripened flavor which may have masked the incipient stages of specific flavor defects.

The results in Table VIII suggested that a longer period of observation at 21 degrees C. might give more accurate information on the keep-

TABLE VIII

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. Days	Defect	15° C. Days	Defect	5° C. Days	Defect	0° C. Days	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	sl. 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	------

sl.—slgthly

------—flavor satisfactory

ing quality of unsalted butter held at lower temperatures. Flavor defects did not appear within seven days at room temperature 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 degrees 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 IX was carried out to show the value of an extended period of observations at room temperature for the prediction of keeping quality at lower temperatures. A new series of 10 churnings of unsalted butter were sampled and one lot from each churning was

TABLE IX

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	Defect	Days	Defect	Days	Defect	Days	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

placed at each of the different temperatures. The periods of examination were as follows:

4, 7, and 10 days at 21 degrees C. (room temperature)
4, 7, 10, and 14 days at 15 degrees C.
7, 14, 21, 28, and 60 days at 5 degrees C.
14, 21, 28, and 60 days at 0 degrees C.

The data recorded in Table IX includes 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 degrees C. showed flavor defects after 14 days and five of the corresponding lots became defective within 10 days at room temperature. Seven of the lots held at 5 degrees C. showed flavor defects after 21 to 28 days and five of the corresponding lots held at room temperature became defective within ten days. Five of the lots held at 0 degrees C. showed flavor defects at the 60 days examination and four of the corresponding lots became defective within 10 days when held at room temperature.

The extension of the period of observation to 10 days at room temperature gave added information on keeping quality as indicated by the closer agreement of the results at this temperature with the results at lower temperatures. In three of the five lots which deteriorated when held at room temperature, the flavor defect was not detected until the 10-day

examination. This indicates that flavor deterioration in unsalted butter held at room temperature occurred too slowly to be detected regularly within seven days,

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

It is of interest to note that lipolytic and proteolytic bacteria were not found on the plates made from salted butter and no definite flavor deterioration occurred within the periods of observation used in this study.

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 II except that non-culture butter was used. The butter was made in 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 34 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 include 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 Februray 1, while the first group was made between September 1 and December 1.

Comparison of the Changes in Numbers of Bacteria in Salted and Unsalted Butter at Different Temperatures—Fig. 2 presents a summary of the changes in numbers of bacteria in salted and unsalted butter held at the different temperatures.

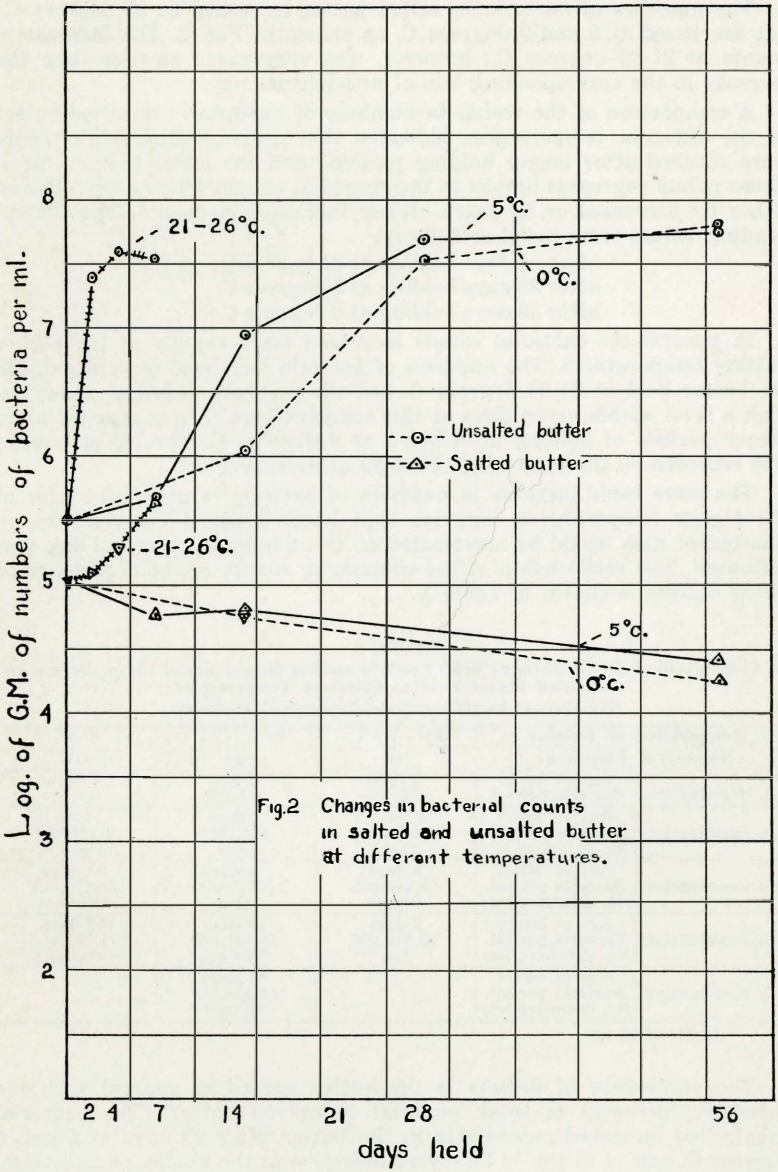

Fig.2 Changes in bacterial counts in salted and unsalted butter at different temperatures.

The numbers of bacteria in salted butter increased at 21 degrees C., but decreased at 5 and 0 degrees C. as shown in Fig. 2. The increase in counts at 21-26 degrees C., however, was very much smaller than the increase in the corresponding lots of unsalted butter.

A comparison of the trends in numbers of bacteria in unsalted butter 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 degrees C.
after 28 days holding at 5 degrees C.
after 28 days holding at 0 degrees 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 21-26 degrees C. but the numbers failed to reach as high a level within seven days at this temperatures as was reached after longer periods of holding at either 5 or 0 degrees C. The highest count was recorded on the butter held 56 days at 0 degrees C.

The more rapid increase in numbers of bacteria in unsalted butter at the higher temperatures suggests that bacteriological deterioration in this butter also 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 X.

TABLE X
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
	No. 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
	No. 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
	No. defective lots	ten	fourteen	sixteen
5th examination	Age of butter		56 days	
	Bacteria per ml.		51,130,000	
	No. defective lots		sixteen	

* G. M. of 15 lots

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 degrees C. and 14 of the 16 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 16 lots became defective within the four-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 XI. In general the numbers of lipolytic bacteria

TABLE XI
Numbers of Lipolytic Bacteria in Unsalted Butter Held at Different Temperatures

Temperature of holding	Time of plating	Number of lots	Range of 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

increased at each of the holding temperatures but the rate and extent of the increases varied considerably. The increases at 5 degrees C. were more extensive than at either 21 to 26 degrees C. or at 0 degrees C. The numbers of lipolytic bacteria exceeded 1,000,000 per ml. in 81.2 per cent of the lots held at 5 degrees C., while 56.2 per cent of the lots held at 0 degrees C. reached counts of over 1,000,000 per ml. The numbers of lipolytic bacteria exceeded 1,000,000 per ml. in only 12.5 per cent of the lots held at 21 to 26 degrees C.

TABLE XII
Numbers of Proteolytic Bacteria in Unsalted Butter Held at Different Temperatures

Temperature of holding	Time of plating	Number of lots	Range of 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 XII 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 degrees C. than at 21 to 26 degrees C., as indicated by the fact that at either 5 or 0 degrees 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 degrees C. developed over 1,000,000 proteolytic bacteria per ml.

Neither lipolytic nor proteolytic bacteria were found in the salted butter corresponding to the unsalted lots noted above. Flavor defects such as were noted frequently in the unsalted butter were absent from the salted butter in these trials.

Comparative Time Required for Flavor Defects to Develop in Unsalted Butter Held at Different Temperatures.—Table XIII gives a summary showing the comparative flavor deterioration in unsalted butter held at different temperatures. The data includes the first flavor defect detected and the final flavor defect noted on each lot. Of the 14 churnings which showed flavor defects within 28 days at 5 degrees C. only nine became

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

Churning no.	Flavor comments on butter					
	21° - 26° C.		5° C.		0° C.	
	Days	Defect	Days	Defect	Days	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	7	------	28	sl. rancid	28	sl. rancid
			56	sl. cheesy	56	sl. rancid
29	7	sl. fermented	56	sl. rancid	56	sl. cheesy
30	7	rancid	14	sl. rancid	28	sl. 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	7	sl. cheesy	28	fruity, rancid	28	sl. rancid
			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	7	------	28	fruity, rancid	28	sl. rancid
			56	cheesy	56	cheesy
37	7	------	28	cheesy	28	sl. off
			56	fruity, rancid	56	fruity, rancid
38	7	------	28	sl. cheesy	28	cheesy
			56	cheesy	56	cheesy
39	4	sour	7	cheesy	14	unclean
	7	cheesy	14	cheesy	28	sl. rancid
40	7	------	14	sl. cheesy	28	sl. off
			28	sl. cheesy, unclean	56	cheesy, putrid
41	4	sour	14	rancid	28	sl. cheesy
	7	sour	28	cheesy	56	cheesy

------—Flavor satisfactory
sl—Slightly

TABLE XIV

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

Churning no.	Flavor comments on butter					
	21° - 26° C.		5° C.		0° C.	
	Days	Defect	Days	Defect	Days	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
sl—Slightly

defective in flavor within seven days at room temperature (21 to 26 degrees C.). The two churnings which kept at 5 degrees C. for 28 days failed to keep for seven days at room temperature. There was some indication that the unsalted butter which deteriorated within seven days when held at room temperature showed flavor deterioration sooner when held at 5 degrees C. than the churnings which kept at the higher temperature.

Of the 13 churnings which showed flavor deterioration within 28 days at 0 degrees C., only eight became defective in flavor within seven days at

room temperature. The three churnings which kept at 0 degrees C. for 28 days failed to keep seven days at room temperature. There was no apparent difference in keeping quality at 0 degrees C. between the churnings which kept and those which failed to keep for seven days at room temperature.

The results in Table XIII show that in certain cases flavor defects did not appear in unsalted butter held at room temperature 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 room temperature suggested that additional time might bring out defects which were probably being overlooked.

The results in Table XIV include observations on a new series of 18 churnings of unsalted butter made without butter culture. Flavor examinations were made at the following periods:

4, 7, and 10 days at room temperature (21 to 26 degrees C.)

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

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

Pronounced flavor deterioration was evident within 28 days in the unsalted butter at either 5 or 0 degrees C. In general, the flavor defects were more pronounced and appeared sooner at 5 degrees C. than at 0 degrees C. Of the 16 lots which deteriorated at both 5 and 0 degrees C., 11 showed flavor defects within 10 days at room temperature. In two churnings flavor deterioration in unsalted butter did not occur at any of the temperatures used. Flavor deterioration in unsalted butter held at 5 and 0 degrees C. but failure to show deterioration at room temperature did not insure good keeping quality at the lower temperatures in every case.

General Conclusions

1. A "holding test" consisting of holding small portions of unsalted butter for seven to 10 days at room temperature (21 degrees 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 degrees 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 degrees 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 degrees or 0 degrees 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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