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Effect of growth of microorganisms on acid numbers of fat in cream and butter

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NOTE TO USERS

EFFECT OF GROWTH OF MICROORGANISMS ON ACID NUMBERS OF FAT IN CREAM AND BUTTER

By

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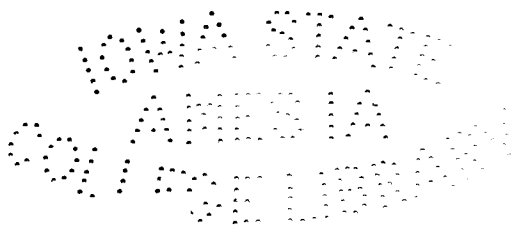

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INTRODUCTION

Changes occurring in the fat of butter are of great importance from the standpoints of flavor and keeping quality of the product. Hydrolysis of the fat may set free some of the lower fatty acids, particularly butyric, caproic and caprylic, causing a condition commonly referred to as rancidity, constituting one of the most serious defects occurring in butter. Rancidity frequently develops in samples of commercial butter when they are subjected to keeping quality tests.

In butter made from unheated cream the lipase normally present in milk may cause fat hydrolysis. However, this enzyme is readily destroyed by the usual pasteurization procedure. Since there is little opportunity for significant recontamination of pasteurized cream or of butter with lipase, it probably has little effect on the keeping qualities of commercial butter.

Many microorganisms are able to hydrolyze butterfat. Organisms of this type are widespread in nature, often being present in raw cream, water and dairy plant equipment. They are ordinarily killed by pasteurization but recontamination after pasteurization may occur. If such organisms gain entrance to pasteurized cream in sufficient numbers and find conditions suitable for growth, they may cause serious defects in the resulting butter. Salt retards the growth of these organisms, so that they produce the most serious defects in unsalted butter.

The acid number has proved to be a valuable adjunct to the organoleptic

method of determining the degree of hydrolysis in the fat of cream or butter. However, certain organisms may utilize fatty acids as food. Also, when the cream acidity is standardized the acids in the fat as well as those in the serum are partially neutralized. Under such conditions the acid number is not an exact index to the degree of fat hydrolysis.

STATEMENT OF PROBLEM

The work herein reported was undertaken to obtain information concerning the effect of growth of microorganisms on acid numbers of fat in cream and in butter. In the studies the following points were considered:

I. Relationship of acid number variations to the qualities and flavor defects of commercial butter.

II. Factors responsible for variations in the acid numbers of the fat in cream and in commercial butter.

III. Relation of volatile acidity of butterfat to rancidity.

IV. Changes in the acid number of butterfat during storage.

HISTORICAL

In the latter part of the nineteenth century butter defects were of considerable interest in Germany, Denmark, Switzerland and other European countries. Much work was done in an effort to ascertain the cause of rancidity and to find a preventive for it. This particular field was of especial importance at that time chiefly because the manufacture of butter on a commercial scale was in its infancy and was beset with many problems, one of the most important being keeping quality. The development of rancidity was probably the most common defect of butter and the most serious problem confronting the butter industry. The early studies included little on the acidity of butterfat but since increased acid numbers most always accompany hydrolytic rancidity in butterfat, a review of the literature concerning rancidity is pertinent.

The early literature is somewhat confusing because of the multiplicity of defects occurring in butter and the lack of clear, concise statements as to the exact nature of the defects studied. This confusion is largely the result of the different conceptions of various workers of the condition known as rancidity.

Workers in the dairy industry commonly use the term rancidity to designate specifically the condition resulting from hydrolysis of the fat. Hagemann (16) believed that rancidity was the result of the splitting of the glycerides of the fatty acids with liberation of butyric acid which was

responsible for the odor of rancid butter. He reported that when lactic acid was mixed with butter, the butter became rancid quickly. From this finding he concluded that the natural formation of lactic acid in dairy products might be responsible for the development of rancidity. He noted also that the glycerol content of rancid butter was less than of fresh butter and suggested that the decomposition of glycerol might be responsible for the off-flavor. Duclaux (13) thought that rancidity was the result of an oxidation process in which the higher acids were oxidized to the lower ones.

The term rancidity was accepted by Browne (6) to imply the condition resulting from any change in the character of the fat and not just the development of free acid. His data reveal that fats may become rancid without necessarily becoming very acid. He further believed that rancidity in butter was primarily the result of the activity of bacteria in the whole butter, with its lactose, casein and other constituents serving as bacterial food. He did not believe, however, that pure fat supported microorganic life. He considered unwarranted the statement often made that rancidity in whole butter was produced by the action of a butyric ferment on the fat with the formation of butyric acid. He thought it more likely that the milk sugar was changed into lactic acid and that the lactic acid was converted into butyric acid causing the characteristic odor of rancid butter. Reinmann (31) studied the development of rancidity in an effort to determine its cause. He found that by inoculating sterile cream with rancid butter he could reproduce the rancid condition. He was unable, however, to reproduce the condition by

inoculation of sterile butter with pure cultures of organisms isolated from rancid butter. He believed that the rancid condition was the result of the action of organisms or enzymes but his work failed to establish either as the specific cause.

The work of Orla-Jensen (28) on the problem of rancidity in butter proved definitely that microorganisms were able to hydrolyze butterfat. He demonstrated that the action of organisms resulted in the liberation of volatile acids which were responsible for the characteristic odor of rancid butter. His data show that Oidium lactis, Cladosporium butyri, Bacterium fluorescens and Bacillus prodigiosus were the most important of the organisms studied from the standpoint of rancidity in butter. In many cases his data reveal that rancidity was accompanied by a decided increase in the acid number of the fat. Contrary to the ideas of many of the earlier workers, he did not believe that light and oxygen were factors in the development of rancidity, except as the oxygen influenced the growth of the organisms splitting the fat. He believed that rancidity could be prevented easily by heating the cream to 85° C., in which process all undesirable organisms are destroyed. He further directed that cream should be cooled and churned in such a manner as to prevent recontamination of the cream and butter with organisms from air, wash water and dairy equipment.

Guthrie (15) believed that actual rancidity in butter seldom occurs and considered that the average person confuses the flavor of strong butter with rancidity. He found that exposure of butter to warmth, light and air did not cause rancidity.

According to Hunziker and Hosman (20), the hydrolysis leading to rancidity is accomplished under ordinary conditions only by fermentation while oxidation is strictly a chemical change.

Mojonnier and Troy (27) believed that the fat of butter becomes rancid as a result of the splitting of the fat molecule and that the fatty acids, when freed from the glycerol radical, have characteristic pungent odors and flavors.

A chemical test for the early detection of rancidity in fats was developed by Kerr (22). This test shows the presence of certain ketones and aldehydes which are known to be present in rancid fat. He suggested that it is especially useful for detecting rancidity in fats which are mixed in such a manner that a slight rancidity might be missed by tasting or smelling.

Kerr and Sorber (23) defined rancidity as a characteristic change which takes place in fats. They stated that the definition was not entirely satisfactory as it included certain conditions which are commonly called rancidity but are not in fact true rancidity. A notable example is the so-called rancidity of butter, the investigation of which has shown it is caused in most cases by a decomposition of milk proteins with or without accompanying hydrolysis of fat. These statements give evidence that the authors were dealing with oxidative changes.

Powick (30) studied the development of rancidity in fats. He indicated that while the term rancidity was sometimes used to describe changes caused by bacteria and fungi in butter, in his studies the term was limited to spoilage that occurred in purified fats under conditions precluding the action

of biological agents. Such a qualifying statement indicates that the work was not concerned with hydrolytic splitting of the fat.

While agreeing with the generally accepted theory that in butter the rancid flavor and odor are due to the presence of free fatty acids resulting from the hydrolysis of fat, Hunziker (19) also suggested that the oxidation of the free fatty acids may play an important role in the production of rancid butter. He further suggested that the free glycerol resulting from the hydrolysis of the fat, which in itself is neutral and free of rancid taste, may yield to oxidation with the formation of acids and aldehydes which have a very pungent odor resembling rancidity.

Grossfeld and Battay (14) found that one part of butyric acid in 12,500 parts of a medium could be detected by the sense of smell.

In general, there are three types of fat deterioration each considered as rancidity, according to Triebold (36) and the end products are quite different. The oxidative rancidity is thought to be due to the addition of molecular oxygen to unsaturated glycerides with the formation of peroxides which subsequently decompose into aldehydes, ketones and fatty acids. In hydrolytic rancidity there is hydrolysis of the glycerides with the liberation of free fatty acids as end products. This type of rancidity is of special interest in spoilage of dairy products due to the liberation of butyric acid with its characteristic odor and taste. The liberation of small amounts of the higher fatty acids does not appreciably affect the odor and taste of a fat. Ketonic rancidity is due to the formation of methyl ketones through the action of certain molds on the lower fatty acids.

In studying the development of rancidity in raw milk when homogenized,

Dorner and Widmer (12) noted a marked difference between this flavor and the flavor obtained when milk becomes rancid without being homogenized. They believed that the rancidity in the unhomogenized milk was more in the odor than in the taste, and that it was an aromatic rancidity which did not have the sharp, bitter taste evident in the homogenized samples. They suggested that this type of rancidity occurred without an appreciable increase in acidity, and that it probably resulted primarily from the breakdown of the glycerides of the volatile acids. The rancidity produced by homogenization was accompanied by a sharp bitter taste, probably as a result of the decomposition of the entire milk fat. Aseptically drawn milk became rancid on homogenizing as rapidly as ordinary milk which indicated that bacteria were probably not a factor in its development. They expressed the opinion that all milk would become rancid in time if it were not for the fact that bacteria cause acidity or spoilage before rancidity occurs.

Collins (9) defined the term rancidity as a condition of fats which is characterized by the odor and flavor of the lower fatty acids, especially butyric acid. He indicated that rancidity is more easily detected by the senses of taste and smell than by chemical means. After examining a large number of samples of commercial butter of varying qualities Clark, et al. (8) concluded that the acid number of the butterfat was a good indication of the quality of the cream from which the butter was made. It was observed that the higher the quality of the butter the lower was the acid number of the fat. These workers apparently did not consider the possibility that the free fatty acids might have been partially neutralized at the time the cream acidity was standardized.

According to Hammer (17) butyric and caproic acids are set free through the hydrolysis of butterfat and a flavor suggestive of these acids sometimes develops in butter and is described as rancid. Microorganisms constitute the most important cause of this hydrolysis. They are widely distributed and are commonly present in raw cream. In general, they are readily destroyed by pasteurization and the defect is easily controlled.

Comparatively little work has been reported concerning the effect of microorganisms on the acid number of butterfat. The work of Browne (6) dealt largely with the oxidative changes occurring in butterfat and he noted that in general as the degree of tallowiness increased the acid number also increased. Seigfeld (34) compared the acid numbers of several samples of fresh sweet and sour cream butter and found no appreciable differences between them. Variations in the acid numbers of fresh sour cream butter were studied by Burr and Weise (7). They found very little variation in the acid numbers throughout the year although the values were lowest between October and February and highest in March and April.

In studying the oxidation of fat, Briggs (5) showed that as oxidation progressed the change in the acid value did not show a close relationship to the absorption of oxygen. He concluded that the acid value does not give a satisfactory means of detecting oxidation.

According to Barnicoat (2) it is generally recognized in scientific literature that no absolute relationship exists between free acid content and rancidity of fats, since rancid fats do not invariably possess a high free acidity.

It is evident that much confusion exists in the literature concerning the definition and causes of the defect known as rancidity. Neither has the relationship of the acid number of butterfat to rancidity been definitely established. The term has often been used without complete enough description of the defect in question to enable a person reviewing the literature to decide definitely the specific nature of the defect referred to.

It is evident that the term rancidity has been used to describe several entirely unlike conditions. One of the common uses of the term was to describe the condition resulting from oxidation of unsaturated fatty acids, a condition referred to today in dairy research as tallowiness. A second use of the term described the condition resulting from hydrolysis of the fat, which today is commonly known in the dairy industry as typical rancidity. Ketonic rancidity is the result of the formation of methyl ketones through the action of certain molds and perhaps other organisms on the lower fatty acids. To further complicate the situation, certain workers in food chemistry, other than dairy products, consider any change occurring in fat as rancidity.

It appears the best criterion to use in determining whether literature concerning rancidity applies to hydrolytic changes in the fat or otherwise is to consider carefully the writer of the article. From a knowledge of the field in which a worker is interested, his interpretation of the term rancidity can usually be determined.

For substantial opinions concerning rancidity in dairy products, its definition and causes, the statements of those workers who have a dairy

viewpoint and who have studied products possessing the defect may be relied upon. The opinions of Orla-Jensen (28), Hunziker (19), Hammer (17), and others can readily be accepted. These men concur in the belief that in general rancidity in butterfat is the result of hydrolytic splitting of the glycerides of the fatty acids in which butyric, caproic and caprylic acids are set free and are responsible for the rancid flavor and odor. Unless qualified, the term rancidity in this thesis will imply changes in the fat of cream or butter resulting in the development of the characteristic flavor and odor of the lower fatty acids. An increase in the acid number of the fat will not be accepted as rancidity, unless accompanied by a rancid odor or flavor.

METHODS

Acid Number of Butterfat

The usual method of expressing the acid number of fat is as the number of milliliters of N/1 alkali required to neutralize the free acid in 100 grams of fat. All references to acid number will imply this meaning. The butter samples were melted, the fat aspirated off and filtered through paper. The samples were melted and filtered in a 45° C. incubator.

The acidity of the fat was determined by the method devised by Breazeale and Bird (4). Ten grams of filtered fat were weighed into a 125 ml. Erlenmeyer flask, 25 ml. of petrolic ether and 10 ml. of absolute ethyl alcohol were added, and the contents of the flask thoroughly mixed. The petrolic ether dissolved the fat and fatty acids and the alcohol dissolved any soap formed during the titration. Ten drops of alcoholic phenolphthalein were added and the sample was titrated against N/10 potassium hydroxide made up in absolute alcohol. The number of milliliters of N/10 potassium hydroxide required to neutralize the free acid in 10 gm. of sample corresponded to the acid number of the fat.

Volatile Acidity of Butterfat

For determining the volatile acidity of fat, 10 gm. of fat were steam distilled under standardized conditions until 200 ml. of distillate were obtained. The distillate was titrated against N/10 aqueous sodium hydroxide

using phenolphthalein as an indicator. The volatile acidity was expressed as the number of milliliters of N/10 sodium hydroxide required to neutralize the first 200 ml. of distillate from a 10 gm. sample of fat.

Types of Cream Used

In studying the effect of selected microorganisms on the acid number of fat in cream and butter, cream of good quality was sterilized in an autoclave after which it was cooled and inoculated with the organisms. The cream was either churned at once or incubated and churned later. The cream was churned and the butter was handled in such a manner that contamination was negligible. In studying the effect of adding lactic acid or certain alkalies to cream on the acid number of fat the same procedure was followed.

In some cases the effect of the normal mixed flora or the lipase of cream was investigated and then raw cream was used.

Organisms Used

The organisms used in the trials were Penicillium roqueforti, Mycoterula lipolytica, Pseudomonas fragi, Pseudomonas fluorescens, Achromobacter lipolyticum, Alcaligenes lipolyticus, Oospora lactis, Lactobacillus bulgaricus, and certain unidentified lipolytic bacilli designated A, B, C, and D; butter cultures were also employed.

SECTION I

RELATIONSHIP OF ACID NUMBER VARIATIONS TO THE QUALITIES AND FLAVOR DEFECTS OF COMMERCIAL BUTTER

Samples of commercial butter of varying qualities were studied to determine any possible correlation between acid number of the fat and quality of the butter. Both unsalted and salted butter were used.

Unsalted Butter

The unsalted butter came from various Iowa creameries and was obtained through a marketing association. Immediately on receipt and after 2, 4 and 6 days at 21° C. the samples were examined for flavor defects. After the 6 days, the fat acid numbers were determined. Whenever sufficient quantities of samples were available, acid numbers of the fat of the fresh butter were also determined.

Table I shows the data on 14 samples of butter. The samples were divided into two groups, those not developing rancidity during holding at 21° C. and those which did. The acid numbers of the fat were not determined in the fresh samples but judging from titrations of many similar samples, it is probable that the original acid numbers were all less than 1.0. In examination of these samples particular attention was given to the detection of rancidity.

In general, the samples which became rancid developed the higher acid

TABLE I

Relationship of Acid Number to the General Quality of Unsalted Butter

Samples from various Iowa Creameries

Sample	Flavor of butter after incubating at 21° C.			Acid number of fat after 6 days at 21° C.
	2 days	4 days	6 days	
Samples not developing rancidity				
1	good	good	good	3.4
2	good	good	good	2.6
3	good	good	good	3.6
4	good	good	good	4.0
5	good	good	good	13.6
6	good	good	good	2.3
7	good	good	good	1.0
8	good	good	good	2.9
9	good	good	good	2.4
Samples developing rancidity				
10	skunk odor	skunk oder	rancid	4.8
11	ester odor*	rancid	v. rancid	6.2
12	ester odor*	rancid	sl. rancid	10.8
13	ester odor*	rancid	rancid	5.6
14	rancid	rancid	rancid	5.8

* The ester odor definitely suggested the odor produced in butter by Ps. fragi.

numbers. Samples 10 to 14 inclusive became rancid and in general the acid numbers were considerably higher than on the non-rancid samples. Sample 5 was an exception having a good flavor and yet having an acid number of 13.6; also samples 1, 3, 4 and 8 had higher acid numbers than are usually found in non-rancid butter.

The results on another series of 32 samples are given in table II. The samples were again divided into those not developing rancidity and those which did. The acid numbers of the fat when the butter was received were below 1.0 in all cases except sample 28, which was 1.1. After 6 days at 21° C., the acid numbers of the non-rancid samples ranged from 0.8 to 11.6; the rancid samples from 2.4 to 14.0. In general, the non-rancid samples had relatively low fat acid numbers, 2, 3, 4, 8, 9, 10, 11, 17, 18, 20 and 24 having acid numbers of 1.0 or slightly higher after 6 days at 21° C. Samples 16 and 25 were exceptions with acid numbers of 9.8 and 11.6 respectively. Of unusual interest were samples 28 and 31. Neither sample was rancid when received but within 2 days sample 28 became slightly rancid and only increased from the original acid number of 1.1 to 1.6. Sample 31 became distinctly rancid during the same period and only increased from 0.8 to 1.3.

It may be noted in the samples developing rancidity after holding, that other flavor defects frequently preceded the rancid odor and flavor. In some cases an ester odor was the first indication of the approach of rancidity. The ester odor definitely suggested the odor produced in butter by Ps. fragi. In every sample in which the ester odor was present, rancidity soon followed. A cheesy flavor occasionally preceded rancidity; however, all samples showing cheesy flavor did not become rancid during the 6 day holding period.

TABLE II

Relationship of Acid Number to the General Quality of Unsalted Butter

Samples from various Iowa Creameries

Sample	Flavor of butter after incubating at 21° C.			Acid number of fat	
	2 days	4 days	6 days	fresh	after 6 days at 21° C.
Samples not developing rancidity					
1	cheesy	cheesy	cheesy	0.7	1.2
2	good	good	good	.7	.8
3	good	good	good	.7	.9
4	good	good	good	.7	.8
5	good	good	good	.6	3.4
6	good	good	good	.7	2.8
7	good	good	good	.6	1.8
8	good	good	good	.6	.9
9	good	good	good	.9	1.3
10	good	good	good	.6	1.1
11	good	good	good	.5	1.0
12	good	good	good	.7	1.8
13	good	good	good	.8	2.2
14	good	good	good	.7	1.6
15	good	good	good	.7	3.1
16	good	good	good	.7	9.8
17	good	good	good	.7	1.0
18	good	good	good	.6	1.0
19	good	good	good	.6	1.4
20	good	good	good	.7	1.0
21	cheesy	cheesy	cheesy	.7	1.5
22	good	good	good	.8	1.8
23	good	good	good	.7	1.4
24	good	good	good	.7	1.0
25	good	good	good	.6	11.6
Samples developing rancidity					
26	good	good	sl. rancid	.7	2.8
27	good	good	sl. rancid	.6	3.2
28	sl. rancid	rancid	rancid	1.1	2.4
29	good	good	sl. rancid	.7	14.0
30	cheesy	sl. rancid	sl. rancid	.8	4.8
31	sl. rancid	rancid	rancid	.8	7.6
32	sl. rancid	sl. rancid	sl. rancid	.6	5.2

These contrasting conditions in which some of the non-rancid samples had high acid numbers and some rancid samples had low acid numbers agree with the findings of Reimmann (31), Guthrie (15) and Barnicoat (2), that there is little correlation between the acid number of butterfat and the development of rancidity. It is possible that some lipolytic organisms have a selective action on certain of the glycerides of the fatty acids. In the one case only the higher acids may be liberated which yield increased acid numbers on the fat without causing serious off-flavors. Other organisms may liberate primarily the lower fatty acids including a small quantity of butyric acid, which while insufficient to increase the acid number, may cause a rancid flavor. Results reported later show that certain organisms, particularly O. lactis, when growing in cream or butter liberate only a very small amount of volatile acid from the fat. This may be a selective action on the fats or the lower acids may be consumed by the growing cells as rapidly as they are liberated as suggested by Orla-Jensen (28).

Salted Butter

The salted butter came from various scoring contests and exhibits and in general was two or more weeks old when received.

The data in table III show the acid numbers of 8 samples of fine quality, lightly salted contest butter scoring 92 to 95. These samples were obtained from creameries submitting entries in the 1938 National Cold Storage butter contest. The acid numbers of the samples ranged from 0.5 to 0.8 and were

TABLE III

Relationship of Acid Number to the General Quality of Salted Butter

Samples entered in the 1938 National Cold Storage Contest

Sample	Score	Acid number
1	94	0.6
2	92	.6
3	92	.5
4	92	.6
5	95	.8
6	94	.6
7	93	.6
8	94	.7

little different from the acid numbers of fat of average quality commercial salted butter as shown in table IV.

Table IV shows the acid numbers of the fat of 27 samples of butter from an Iowa State College Educational Butter Scoring Contest. Some of these samples were made from neutralized cream. The scores ranged from 90 to 93 and the acid values from 0.5 to 0.8. Judging from the scores of the lots of butter there must have been considerable difference in the qualities of the cream from which they were made. It appears that there was little correlation between acid numbers of the fat of neutralized cream butter and the quality of the cream from which it was made. In fact sample 1, a 93 score butter, had an acid number of 0.8 while sample 3, a 90 score butter, had an acid number of 0.6.

The data shown in table V indicate that Oklahoma butter exhibited at the Oklahoma State Fair had slightly higher fat acid numbers than Iowa butter of similar quality. The exact age of these samples and the conditions under which they were manufactured were not known. Possibly the age and quality of the cream when churned, age of the butter and period of lactation of the producing cows may have influenced the acid values.

In reviewing the studies on unsalted and salted commercial butter of widely varing qualities, certain observations should be pointed out. There was no definite relationship between the acid number of the fat of unsalted butter and flavor defects. The fat of fresh unsalted butter invariably had low acid numbers and increases after holding 6 days at 21° C. were always evident. About 25 per cent of the samples of unsalted commercial butter

TABLE IV

Relationship of Acid Number to the General Quality of Salted Butter

Samples from Iowa State College Educational Butter Scoring Contest - some made from neutralized cream

Sample	Origin of butter	Score	Acid number
1	Iowa	93.0	0.8
2	Iowa	92.5	.7
3	Texas	90.0	.6
4	Iowa	91.5	.7
5	Iowa	92.0	.5
6	Iowa	91.5	.8
7	Iowa	92.0	.7
8	Oregon	91.0	.8
9	Nebraska	91.5	.7
10	Iowa	91.5	.7
11	Iowa	92.0	.6
12	Iowa	93.0	.7
13	Iowa	90.0	.7
14	Iowa	90.5	.7
15	Iowa	91.0	.5
16	Iowa	91.0	.7
17	Iowa	91.0	.5
18	Iowa	91.0	.8
19	Iowa	91.5	.7
20	Iowa	91.5	.6
21	Iowa	90.5	.5
22	Iowa	91.0	.7
23	Iowa	91.0	.7
24	Iowa	91.0	.6
25	Iowa	90.0	.6
26	Iowa	90.5	.6
27	Iowa	90.5	.7

TABLE V

Relationship of Acid Number to the General Quality of Salted Butter

Samples exhibited at the 1938 Oklahoma State Fair

Sample	Score	Acid number
1	92.0	1.4
2	93.0	.7
3	92.5	.6
4	93.0	.8
5	90.5	.5
6	92.0	.8
7	92.0	1.0
8	89.0	2.2
9	93.0	.6
10	92.0	.6
11	92.0	.6
12	89.0	1.8
13	89.5	.9
14	92.5	.6
15	90.0	.7
16	93.0	.5
17	90.0	.8
18	89.5	.5
19	88.5	1.2
20	91.5	1.7
21	90.0	.9
22	90.0	1.0
23	90.0	1.0
24	90.5	.9
25	91.0	.9

observed became rancid during the holding period. In the samples not developing rancidity with a few exceptions, the increases in the acid numbers were small. In the samples developing rancidity however, the increases were significant although exceptions to this generalisation were also encountered. Some samples of unsalted butter of good flavor had very high acid numbers after the holding period while certain rancid samples had low acid numbers.

Many samples of salted butter were subjected to keeping quality tests but since very few of the samples became rancid, acid values were not determined after the holding period. The acid numbers of the fat of fine quality, lightly salted contest butter were similar to those of commercial salted butter of considerably lower quality. There was no correlation between the scores of salted butter and the acid numbers of the fat.

SECTION II

FACTORS RESPONSIBLE FOR VARIATIONS IN THE ACID NUMBERS OF THE FAT IN CREAM AND IN COMMERCIAL BUTTER

Effect of the Normal Mixed Flora and Milk Lipase in Raw Cream on the Acid Number of the Fat

Raw cream from several sources was used in the trials to determine the degree of hydrolysis caused by lipolytic organisms and by lipase in cream. Palmer (29) reported that 1 part formaldehyde in 1500 parts of cream inhibited the growth of most organisms with no detrimental effect on the milk lipase*. In order to determine the effect of formaldehyde on pure cultures of some of the lipolytic organisms commonly present in raw cream, small lots of sterilized cream were inoculated with known lipolytic organisms. Formaldehyde was immediately added to the cream in concentrations ranging from 1 part in 4800 parts to 1 part in 1200 parts of cream. These lots of cream were churned after holding 6 days at 21° C. The effect of formaldehyde on the organisms was determined by their ability to grow as evidenced by increases in the acid number of the fat.

Organisms varied considerably in their tolerance for formaldehyde (table VI). Ach. lipolyticum, Mye. lipolytica and Ps. fluorescens grew very little in a concentration of 1 part formaldehyde to 4800 parts of cream, while O. lactis grew luxuriantly in all concentrations up to 1 part in 2000 parts of cream. None of the organisms showed appreciable activity in cream containing 1 part of formaldehyde in 1600 parts of cream. As a result of these trials it was assumed that any lipolysis which occurred in raw cream containing 1 part formaldehyde to 1500 parts cream was due largely to the

* Recent work by B. L. Herrington and V. N. Krukovsky has established the presence of two lipases in milk. One is inhibited completely by small amounts of formaldehyde, the other is not sensitive to moderate amounts of it. J. Dairy Sci. 22 : 127-135, 1939.

TABLE VI

Resistance of Certain Lipolytic Microorganisms to Formaldehyde in Cream

Acid number of original fat 0.6

Concentration of formaldehyde in cream	Acid number of butterfat after incubating cream 6 days at 21° C.			
	Cream inoculated with			
	Ach. lipolyticum	Myc. lipolytica	Ps. fluorescens	O. lactis
0	3.3	80.6	5.0	16.3
1-4800	1.0	1.3	1.5	16.4
1-3600	.9	1.8	1.4	13.3
1-2400	.9	1.2	1.2	7.9
1-2000	.9	1.1	1.1	6.7
1-1600	.9	1.2	1.0	.8
1-1400	.9	1.3	1.0	.7
1-1200	1.0	1.5	1.2	.8

action of milk lipase while in the cream containing no formaldehyde, the lipolysis was due to the combined action of lipase and microorganisms.

Small portions of several lots of raw cream from different sources, with and without formaldehyde added, were stored for 2, 4, 6, 10 and 14 days at 5°, 13° and 21° C. The lots of cream were then churned and acid numbers of the butterfat determined.

The data shown in tables VII, VIII and IX reveal that in general, the lipolysis in the samples containing no formaldehyde was greatest at the lowest temperature. At 21° C. the rate of acid formation was considerably greater than at the lower temperatures which apparently tended to check the growth of some of the lipolytic organisms. Exceptions to this generalization were occasionally encountered, as for example in table VII, the cream stored at 21° C. for 14 days showed a higher fat acid number than did another lot of the same cream stored at lower temperatures. In the samples containing formaldehyde supposedly only the milk lipase was active in splitting the fat. The lipase in these samples caused hydrolysis at all temperatures studied but increases in its activity were evident as the temperature of storage increased. The acid numbers of the fat of one group of these samples containing formaldehyde (table VII) after 14 days storage at 5°, 13° and 21° C. were 6.0, 7.5 and 13.2, respectively. Increases in the titratable acidity of the samples of cream treated with formaldehyde also occurred at all temperatures studied. From the same table it may be observed that from the original titratable acidity of0.16 per cent, the acidity of the samples containing formaldehyde increased in 14 days at 5°, 13° and 21° C. to 0.36, 0.40 and 0.48 per cent, respectively. These increases were probably due to

TABLE VII

Effect of the Normal Mixed Flora and Milk Lipase in Raw Cream on the Acid Number of the Fat

Cream separated from mixed milk of several cows

Days held at	Normal raw cream			Formaldehyde added (1-1500)		
	Per cent acidity	Acid number	Flavor	Per cent acidity	Acid number	Flavor
5° C.						
0	0.16	0.9	good	0.16	0.9	good*
2	.26	6.5	bitter, rancid	.21	3.5	oxidized
4	.35	13.2	bitter, rancid	.24	3.9	oxidized
6	.51	11.1	bitter, rancid	.28	4.5	rancid
10	.60	15.2	bitter, rancid	.28	5.3	rancid
14	.69	17.1	bitter, rancid	.36	6.0	rancid
15° C.						
0	.16	.9	good	.16	.9	good
2	.58	7.6	rancid	.22	4.0	rancid
4	.70	8.2	rancid	.24	4.8	rancid
6	.88	7.6	rancid	.30	5.5	rancid
10	1.00	10.1	rancid	.35	6.2	rancid
14	1.14	11.5	rancid	.40	7.5	rancid
21° C.						
0	.16	.9	good	.16	.9	good
2	.70	6.5	good	.22	4.9	oxidized
4	.70	8.2	very sour	.28	4.8	oxidized
6	.97	9.0	rancid, cheesy	.38	7.5	rancid
10	1.03	11.5	rancid, cheesy	.40	9.5	rancid
14	1.05	22.2	rancid, cheesy	.48	13.2	rancid

* Formaldehyde was detectable in all samples containing it; the term "good" was used to indicate the absence of a rancid flavor or odor.

TABLE VIII

Effect of the Normal Mixed Flora and Milk Lipase in Raw Cream on the Acid Number of the Fat

Cream separated from mixed milk of several cows

Days held at	Normal raw cream			Formaldehyde added (1-1500)		
	Per cent acidity	Acid number	Flavor	Per cent acidity	Acid number	Flavor
5° C.						
0	0.14	0.6	good	0.14	0.6	good*
2	.26	6.5	rancid	.21	3.5	rancid
4	.35	13.2	rancid	.24	3.6	rancid
6	.51	16.0	rancid	.28	4.1	rancid
10	.62	21.1	rancid	.30	5.0	rancid
14	.75	27.0	rancid	.33	6.8	rancid
13° C.						
0	.14	.6	good	.14	.6	good
2	.58	7.6	rancid	.22	4.0	rancid
4	.70	8.4	rancid	.24	4.8	rancid
6	.88	9.1	rancid	.30	5.0	rancid
10	.90	11.2	rancid	.32	6.3	rancid
14	.92	13.1	rancid	.34	7.5	rancid
21° C.						
0	.14	.6	good	.14	.6	good
2	.70	6.5	rancid	.22	4.9	rancid
4	.76	9.6	rancid	.28	5.7	rancid
6	.96	11.0	rancid	.38	6.0	rancid
10	.95	12.3	rancid	.40	7.5	rancid
14	.97	14.5	rancid	.41	9.2	rancid

* See footnote, table VII.

TABLE IX

Effect of the Normal Mixed Flora and Milk Lipase in Raw Cream on the Acid Number of the Fat

Cream separated from mixed milk of several cows

Days held at	Normal raw cream			Formaldehyde added (1-1500)		
	Per cent acidity	Acid number	Flavor	Per cent acidity	Acid number	Flavor
5° C.						
0	0.15	1.4	good	0.15	0.6	good*
2	.15	2.6	good	.16	.6	good
4	.16	2.2	old	.16	.6	good
6	.21	3.8	acid	.17	.6	good
10	.49	5.6	sl. rancid	.17	.9	good
14	.66	6.1	rancid	.17	1.0	good
15° C.						
0	.15	1.4	good	.15	.6	good
2	.47	1.0	bitter, acid	.16	.6	good
4	.63	1.0	acid, bitter	.16	.7	good
6	.70	1.3	acid, rancid	.16	.8	good
10	.80	2.2	acid, rancid	.16	.9	good
14	.74	3.0	acid, rancid	.18	1.0	good
21° C.						
0	.15	1.4	good	.15	.6	good
2	.59	1.0	acid, bitter	.17	.8	good
4	.74	1.3	acid, rancid	.16	.8	good
6	.75	1.5	acid, putrid	.18	1.0	good
10	.85	4.5	acid, putrid	.18	1.6	oxidized
14	.77	6.9	acid, putrid	.22	1.7	oxidized

* See footnote, table VII.

liberation of certain fatty acids by lipase. Increases in the acid numbers of the fat were roughly proportional to the increases in titratable acidity. The increases in acid numbers and titratable acidities were thought to be due largely to the action of milk lipase on the fat since plate counts on these samples revealed relatively few organisms. Rarely did the plates show more than a few hundred organisms per milliliter. Long (26) and Collins (9) reported that organisms must be present in reasonably large numbers to cause defects and it is believed that there were too few organisms in these samples to cause the acidity increases observed. This statement agrees with the findings of Krukovsky and Sharp (24) who showed that raw milk on standing at temperatures too low for bacterial growth, increased considerably in titratable acidity.

In some of the samples containing no formaldehyde marked increases in the acid number of the fat were noted between 10 and 14 days of storage. These sharp increases probably were caused by more rapid mold growth during this period.

The data in table X show that when milk from individual cows was held at 5° C., fatty decomposition occurred due to growth of organisms and action of lipase very similar to that observed in mixed herd milk.

Of the two biological agencies capable of causing lipolysis as measured by the acid number of the fat, the action of microorganisms was of somewhat greater importance than the action of milk lipase. In the raw cream in which both microorganisms and normal milk lipase had been active the acid numbers of the fat were often 3 to 4 times greater than in the samples

TABLE X

Effect of the Normal Mixed Flora and Milk Lipase in Raw Cream on the Acid Number of the Fat

Cream separated from milk of individual cows

Cow	Fresh cream		Cream after 14 days at 5° C.			
			Normal		Formaldehyde added (1-1500)	
	Acid number of fat	Flavor	Acid number	Flavor	Acid number	Flavor
1	0.5	good	4.8	rancid, sour	1.2	rancid
2	.6	good	2.6	rancid, sour	.8	rancid
3	.5	good	2.2	sour	1.9	putrid, sour
4	.5	good	3.1	fair	.9	putrid
5	.6	good	2.0	fair, rancid	1.7	rancid
6	.6	good	1.6	rancid, sour	.9	rancid
7	.7	good	5.0	rancid	3.0	sl. rancid
8	.9	good	2.2	rancid	1.2	sl. rancid
9	.7	good	2.8	rancid	1.5	old
10	.7	good	1.6	rancid	1.0	rancid

containing formaldehyde. This is particularly well demonstrated by the data shown in table VIII. The samples held at 5° C. containing formaldehyde at 4, 6, 10 and 14 days had acid numbers of 3.6, 4.1, 5.0 and 6.8, respectively while similarly held samples of the same cream containing no formaldehyde had acid numbers of 13.2, 16.0, 21.1 and 27.0, respectively. The increases in acid numbers due to growth of microorganisms were much greater than the increases due to milk lipase. The data presented reveal an unusual circumstance in that in every case the total lipolysis at 13° C. was less than at either 5° or 21° C. after 14 days of storage. No explanation is offered for this condition.

Davies (11) reported that certain metals tended to inhibit lipase activity in butter. In order of their inhibiting power were copper, iron, nickel, cobalt, manganese and chromium. Tin and aluminum had no effect. In the trials reported no effort was made to check or control the normal metal contamination. The results obtained may have been influenced by this factor.

Rice and Markley (32) reported that one of the causes of rancidity in dairy products is the carrying over of the enzyme into the manufactured products. In view of the wide use of the pasteurization process for dairy products it seems doubtful that a significant carry-over of lipase would occur under ordinary factory conditions.

From the data presented it may be readily seen that fatty decomposition occurred in cream even when stored at low temperatures. All samples of normal raw cream contained lipolytic microorganisms which were capable of causing hydrolysis of fat if conditions favored their growth. These results

agree with the findings of Hammer and Collins (18) who showed that lipolytic organisms were common in fresh raw milk and cream. A fat splitting enzyme was also present in all samples studied. Both of these agencies were active throughout a wide range of temperature. There was, however, considerably more hydrolysis of the fat at all temperatures in the samples containing no formaldehyde which indicates that microorganisms were active in splitting the fat. While considerable variation may be expected in the degree of fat decomposition in cream from different sources, the importance of procuring and processing cream by the creamery soon after it is produced is emphasized.

Effect of the Growth of Butter Culture Organisms and *L. bulgaricus* on the Acid Number of the Fat of Cream

Most of the butter manufactured in the United States is made from gathered cream, only a comparatively small amount being made from milk separated in creameries. Gathered cream is received by creameries in some areas in a sweet condition while in others it often is excessively sour. In most of the butter producing areas the maximum acidity encountered in cream is 0.8 to 1.0 per cent and this acid is largely the result of the growth of *S. lactis* organisms. In some sections however, particularly in the southwest, cream sometimes develops an acidity considerably in excess of 1.0 per cent. Such an acidity is largely the result of the growth of lactobacilli. Because of poorly organized procurement systems and lax cream grading regulations, cream often remains on farms and in cream stations for prolonged periods before delivery. This situation, coupled with high temperatures, provides conditions suitable for the growth of lactobacilli.

Plant practices often involve the ripening of cream. In this process the acidity may be increased materially before the cream is churned. Under the conditions described the acidity produced is primarily the result of the fermentation of lactose by *S. lactis* which results in the production of lactic acid. The effect of the growth of these homofermentative organisms in cream on the acid number of the fat has been investigated. In trials, portions of sweet cream were sterilized, inoculated with 1 per cent butter

culture and ripened at 21° C. to varying acidities. Other lots of cream were inoculated with cultures of L. bulgaricus and ripened at 37° C. to acidities considerably above 1 per cent. The lots of cream were then cooled, churned and the acid numbers of the fat determined.

The data in table XI show that even though the acidities of the cream were increased to the normal maximum of butter culture organisms, which is considerably above the normal churning acidity, the acid number of the fat was not changed appreciably. Likewise, the data in table XII reveal that the growth of the L. bulgaricus failed to alter the acid number of the fat. These results indicate that growth of the common homofermentative organisms in cream is not responsible for increases in the acid number of butterfat and confirm the findings of Orla-Jensen (28) and of Laxa (25) whose data show that ordinary milk souring bacteria had no influence on the acid number of the fat.

TABLE XI

Effect of the Growth of Butter Culture Organisms on the Acid Number of the Fat of Cream

Cream incubated at 21° C.

TRIAL I		TRIAL II		TRIAL III	
Per cent acidity in cream	Acid number of fat	Per cent acidity in cream	Acid number of fat	Per cent acidity in cream	Acid number of fat
0.11	0.60	0.09	0.55	0.11	0.65
.39	.65	.27	.50	.25	.60
.49	.60	.35	.50	.35	.65
.58	.60	.60	.50	.58	.65
.80	.65	.82	.55	.85	.60
.86	.60	.89	.50	.88	.65

TABLE XII

Effect of the Growth of L. bulgaricus on the Acid Number of the Fat of Cream

Cream incubated at 37° C.

TRIAL I		TRIAL II	
Per cent acidity in cream	Acid number of fat	Per cent acidity in cream	Acid number of fat
0.10	0.70	0.13	0.45
.88	.65	.75	.50
1.25	.65	1.30	.55
1.60	.70	1.52	.45
1.88	.75	2.02	.45

Effect of Adding Lactic Acid to Cream on the Hydrolysis of Fat by Pure Cultures of Lipolytic Microorganisms

Work reported later in this paper indicates that the growth of butter culture organisms resulting in the formation of lactic acid tended to inhibit the lipolytic action of certain of the organisms studied. In those trials the bacterial species studied were far more readily inhibited in their action on fat than were the molds and yeasts.

The question arose as to whether this inhibition was simply the result of the formation of lactic acid in the cream or whether the presence of growing butter culture organisms might have exerted some influence on the oxygen demands or other growth needs of the lipolytic organisms. In order to determine this point, lactic acid was sterilized and added to sterilized cream in such amounts that samples of the same lot of cream were obtained, varying in reaction from sweet in the check sample to very sour in the acidulated samples. These samples of cream were inoculated with various lipolytic microorganisms. After incubating for 6 days at 21° C., the samples were churned and the acid numbers of the fat determined. As a check on the effect of the acid on the acid number of the fat, a series of acidulated but uninoculated samples was held for the same period as the inoculated samples and the acid numbers of the fat determined.

As shown in table XIII, the uninoculated cream ranged in titratable acidity from 0.20 per cent (check sample) to 2.61 per cent in the sample

TABLE XIII

Effect of the Addition of Lactic Acid on the Growth of Lipolytic Microorganisms in Cream

Lot	Cream acidulated — not inoculated		Cream acidulated and incubated 6 days at 21° C. after inoculation with					
			O. lactis		Mye. lipolyticum		Ach. lipolyticum	
	Per cent acidity	Acid number of fat	Per cent acidity	Acid number of fat	Per cent acidity	Acid number of fat	Per cent acidity	Acid number of fat
1	0.20	0.5	0.82	17.5	0.85	20.5	0.39	9.2
2	.22	.5	.43	11.0	.47	14.6	.31	3.1
3	.45	.5	.52	12.8	.63	10.9	.38	2.8
4	.62	.5	.59	3.2	.72	5.6	.47	2.7
5	.87	.5	.66	2.7	.81	3.7	.76	1.6
6	1.15	.5	.90	1.5	1.05	2.6	1.02	.6
7	1.66	.5	1.38	1.0	1.53	1.5	1.51	.6
8	2.61	.5	2.20	.8	2.25	1.0	1.98	.6

receiving the largest portion of added lactic acid. No differences were observed in the acid numbers of the fat of this entire series of cream samples after incubation. These data indicate that lactic acid, even in concentrations greater than normally formed in cream, did not cause an increase in the acid number of fat. These results are confirmed by similar data shown in table XIV.

O. lactis when inoculated into sweet or moderately sour cream increased the titratable acidity appreciably probably due to the liberation of acids from the fat. As the amount of added lactic acid was increased the action of the molds on the fat decreased. The samples of cream having titratable acidities of less than approximately 0.50 per cent when inoculated, showed increases in titratable acidity due to the growth of the mold; samples having titratable acidities over approximately 0.50 per cent when inoculated, showed decreases. In general, the lower the titratable acidity of the cream when inoculated with O. lactis, the higher the acid number of the fat became, due to mold growth. From the data (table XIV) it may be seen however that marked increases in the acid number of the fat were observed even when the mold was inoculated into cream having titratable acidities in excess of 1.0 per cent.

Mya. lipolytica when inoculated into samples of cream having varying acidities due to added lactic acid apparently grew luxuriantly even in the samples having very high titratable acidities. In table XIV it may be observed that a marked increase in the acid number of the fat occurred when cream having an acidity of 2.08 per cent was inoculated with this

TABLE XIV

Effect of the Addition of Lactic Acid on the Growth of Lipolytic Microorganisms in Cream

Lot	Cream acidulated — not inoculated		Cream acidulated and incubated 6 days at 21° C. after inoculation with					
			O. lactis		Mye. lipolytica		Ash. lipolyticum	
	Per cent acidity	Acid number of fat	Per cent acidity	Acid number of fat	Per cent acidity	Acid number of fat	Per cent acidity	Acid number of fat
1	0.18	0.5	0.48	26.1	0.60	----	0.41	11.3
2	.24	.7	.29	19.6	.46	----	.41	8.9
3	.41	.5	.45	15.8	.52	27.8	.49	7.8
4	.52	.7	.45	18.4	.60	21.8	.55	7.8
5	.71	.5	.55	14.0	.74	21.4	.66	5.0
6	.97	.5	.60	8.5	.85	13.7	.88	6.4
7	1.34	.5	.86	4.7	1.12	12.8	1.36	1.0
8	2.08	.6	1.34	3.7	1.72	3.1	2.17	.9

organism. In the cream having acidities above approximately 0.70 per cent, the organisms apparently utilized the acid in their growth since decreases in titratable acidities were observed in these samples.

Ach. lipolyticum formed some acid when growing in cream and in some of the trials was able to hydrolyze fat when inoculated into cream having a titratable acidity of about 1.0 per cent (table XIV) as is evidenced by increased acid numbers of the fat in these samples.

The organisms studied were types which are commonly found in cream, and it seems that it is not safe to assume the undesirable types of organisms will be controlled in cream having high titratable acidity. It can readily be seen that any of the organisms studied might cause appreciable damage to the quality of cream even though it was sour. Lactic acid in cream in quantities greatly exceeding the amount normally produced by ordinary milk souring organisms, was definitely not a factor contributing to increased acid numbers in butterfat.

Effect of Lactic Acid Production in Cream on the Hydrolysis of Fat by Microorganisms

It has been reported that the development of lactic acid in milk or cream has a restraining influence on the growth of undesirable types of microorganisms. To study this problem, sterilized cream was inoculated at approximately the same time with a butter culture and a culture of a common lipolytic microorganism. Lipolytic molds, yeasts and bacteria were used in these trials and included O. lactis, Myc. lipolytica, Ach. lipolyticum, Alc. lipolyticus and Ps. fluorescens. After inoculation, the lots of cream were held at 21° C. The acidity and flavor of the cream and acid number of the fat were determined after 2, 4 and 6 days.

After 6 days incubation at 21° C. (table XV) the fat in the sample inoculated only with the mold had an acid number of 40.4 while the sample inoculated with butter culture organisms as well as O. lactis had an acid number of 10.4. It is very evident that the growth of butter culture organisms with the resultant formation of lactic acid inhibited the lipolytic activity of O. lactis in cream.

After 6 days incubation the sample containing only Myc. lipolytica had a fat acid number of 37.9 while the acid number of the fat in the sample inoculated with butter culture organisms as well as the yeast was 47.9. This indicates that the lactic organisms growing in the cream were really an incentive for increased lipolytic action by the yeasts.

TABLE XV

Effect of the Growth of Butter Culture Organisms in Cream on the Growth of Lipolytic Organisms

Days held at 21° C.	BUTTER CULTURE ADDED: Per cent acidity	Acid number	Flavor of cream	NO BUTTER CULTURE ADDED: Per cent acidity	Acid number	Flavor of cream
O. lactis						
2	0.86	10.9	acid, rancid	0.17	12.5	fruity
4	.81	6.5	acid, rancid	.22	24.8	rancid, fruity
6	.77	10.4	acid, rancid	.24	40.4	rancid, fruity
Myc. lipolytica						
2	.86	2.7	rancid	.23	8.8	rancid
4	1.04	20.1	bitter, rancid	.38	20.1	rancid, bitter
6	.98	47.9	bitter, rancid	.38	37.9	rancid, bitter
Agh. lipolyticum						
2	.76	1.8	acid, old	.28	2.4	old
4	.95	2.1	acid, old	.31	4.8	acid, rancid
6	.96	2.5	acid, old	.37	8.3	rancid
Alc. lipolyticus						
2	.81	.5	acid	.19	.8	good
4	.87	1.0	acid	.23	2.4	old
6	.88	1.6	acid	.23	2.6	sl. rancid
Ps. fluorescens						
2	.88	1.4	acid, unclean	.19	1.4	rancid
4	.92	2.0	acid, unclean	.27	3.6	rancid, putrid
6	.96	2.5	acid, old	.27	4.4	rancid, putrid

After 6 days incubation, the acid numbers of the fat of cream inoculated with cultures of lipolytic bacteria, with and without butter cultures respectively, were as follows; Ach. lipolyticum 2.5 and 8.3; Alc. lipolyticus 1.6 and 2.5 and Ps. fluorescens 2.5 and 4.4. All three species of lipolytic bacteria used in this study were definitely inhibited in their activity by the growth of butter culture organisms.

In general the results obtained in these trials agreed quite closely with the results obtained when pure lactic acid was added to cream containing these organisms. The one exceptional organism was Myc. lipolytica which was definitely inhibited by acidulating the cream to 0.50 to 0.60 per cent. When grown in combination with butter culture organisms, its growth was definitely accelerated even when the acidity reached 0.98 per cent. Further work should be done to determine the significance of these results.

Effect of Growth of Certain Lipolytic Organisms in Cream and Butter on the Acid Number of the Fat

Several organisms which showed definite lipolysis when grown on an agar medium containing fat emulsion were inoculated into portions of sterilized cream. After 7 days incubation at 21° C. the cream samples were churned and the acid numbers of the fat determined.

While all of the organisms showed definite lipolysis on agar plates (table XVI), some of them failed to produce rancidity in cream or butter or to cause marked increases in the acid number of the fat. Some of the organisms caused increases in the acid number of the fat and yet failed to produce a typically rancid odor.

Hammer and Collins (18) reported that the highest lipolytic counts were secured on butter that was cheesy rather than rancid. Long (26) also found that certain cultures showing lipolysis on plates containing fat often failed to produce rancidity in butter. Various flavors were produced by the organisms studied, including old, acidy, roquefort, putrid, cheesy and rancid. The acid numbers on the fat of the inoculated samples after incubation ranged from 0.6 to 16.8.

Orla-Jensen (28) showed that certain organisms were able to bring about rancidity and cause high acid values on the fat. In working with pure cultures of Ps. fragi, Hussong (21) found that this organism was quite actively lipolytic and caused an increase in the acid number of the fat

TABLE XVI

Effect of Growth of Lipolytic Organisms in Cream on the Acid Number of the Fat

Cream incubated 7 days at 21° C. after inoculation	Per cent acidity in cream	Acid number of fat	Flavor of cream
P. roqueforti	0.32	8.6	roquefort
Myc. lipolytica	1.57	12.4	acid, yeasty
Ps. fragi	.25	.6	old
Ps. fluorescens	.50	9.5	old, putrid
Ach. lipolyticum	.39	9.4	putrid, rancid
Alc. lipolyticus	.43	16.8	cheesy, rancid
O. lactis	.48	10.1	acidy
Unidentified bacillus A	.30	1.8	putrid
Unidentified bacillus B	.53	3.5	putrid
Unidentified bacillus C	.32	3.4	putrid, rancid
Unidentified bacillus D	.37	1.9	roquefort, rancid
Cream before sterilization	.16	.8	good
Cream after sterilization	.15	.7	good, heated

which was accompanied by a rancid flavor and odor. His work also showed that certain organisms caused marked increases in the acid number of the fat but failed to produce a rancid oder and that many lipolytic organisms are also proteolytic.

It has been shown by many workers that certain microorganisms have the ability to hydrolyze fat when growing in cream and in butter. A series of trials were made to determine the comparative lipolytic activity of several organisms when growing in cream and in butter. Sweet cream was sterilized in an autoclave, cooled to 21° C., and inoculated with a culture of the organism under consideration. The inoculated cream was well mixed and divided into two portions. One of these portions was churned, the butter was packed in sterile containers and placed in storage at 5°, 13° and 21° C. The other portion of the inoculated cream was carefully transferred to sterilized fruit jars and placed in storage at the same temperatures as the butter. After 4, 6, 10 and 14 days of storage, samples of cream and butter were removed from storage. The cream was churned and flavor of the butter and acid number of the fat of each sample were determined. In addition the titratable acidity of the cream was determined.

The data showing the lipolytic activity of Ach. lipolyticum is presented in table XVII. The acid numbers on both the fat of cream and of butter increased progressively throughout the 14 day period. In general the growth at all temperatures was more rapid in cream than in butter as is evidenced by the greater acid numbers on the fat of cream than on the fat of butter under the same holding conditions. The acid numbers in both

TABLE XVII

Comparative Lipolytic Action of Ach. lipolyticum in Cream and in Butter

Days held at	Cream			Butter	
	Per cent acidity	Flavor	Acid number	Flavor	Acid number
5° C.					
0	0.13	good	1.0	good	1.0
4	.30	good	1.3	good	1.3
6	.31	old	2.0	old	1.4
10	.32	old	2.7	rancid	1.6
14	.32	sl. rancid	3.1	sl. rancid	1.7
13° C.					
0	.13	good	1.0	good	1.0
4	.31	good	2.0	good	1.6
6	.34	old	4.3	off-flavor	1.8
10	.36	sl. rancid	4.9	rancid	2.4
14	.35	sl. rancid	5.9	rancid	3.1
21° C.					
0	.13	good	1.0	good	1.0
4	.32	old	2.8	good	1.9
6	.33	old	3.3	sl. rancid	2.7
10	.41	rancid	6.2	rancid	3.5
14	.40	rancid	7.1	rancid	4.5

cream and butter increased most rapidly at the higher temperatures, being at the end of 14 days in the cream 3.1, 5.9 and 7.1 when held at 5°, 13° and 21° C., respectively, and in the butter under the same holding conditions the acid values were 1.7, 3.1 and 4.5. This organism formed comparatively little acid at any temperature, 0.41 per cent being the maximum. In general, off-flavors were evidenced in the cream and butter after about the same period of storage at each temperature regardless of differences in acid numbers.

The results with Myc. lipolytica (table XVIII) were similar to those obtained with Ach. lipolyticum with the exception that greater increases in the acid numbers of the fat were observed at all temperatures and all storage periods. After 14 days the acid numbers of the fat of the cream were 34.0, 39.7 and 42.0 at 5°, 13° and 21° C., respectively, and in similarly handled butter the corresponding values were 7.0, 27.9 and 32.6.

O. lactis grew more luxuriantly in cream than in butter at all temperatures. This greater growth was evidenced by larger acid numbers on the fat of cream as shown in table XIX. After 14 days the acid values in cream were 15.5, 19.4 and 43.0; in butter the corresponding values were 4.1, 14.8 and 38.5. This organism caused greater fatty breakdown in both cream and butter at all temperatures than did the Ach. lipolyticum but did not cause as much fat hydrolysis as Myc. lipolytica at 5° or 13° C.

The data presented substantiate the statement that the organisms studied, which included common bacterial, mold and yeast species, grew more luxuriantly in cream than they did in butter. The differences were

TABLE XVIII

Comparative Lipolytic Action of Myc. lipolytica in Cream and in Butter

Days held at	Cream			Butter	
	Per cent acidity	Flavor	Acid number	Flavor	Acid number
5° C.					
0	0.12	good	0.9	good	0.9
4	.16	good	1.0	good	1.8
6	.17	good	3.0	good	3.3
10	.19	rancid	11.7	sl. rancid	5.2
14	.23	v. rancid	34.0	v. rancid	7.0
15° C.					
0	.12	good	.9	good	.9
4	.21	old	7.4	sl. rancid	9.9
6	.27	old	13.8	rancid	14.5
10	.31	v. rancid	18.3	v. rancid	21.0
14	.35	v. rancid	39.7	v. rancid	27.9
21° C.					
0	.12	good	.9	good	.9
4	.24	sl. rancid	7.0	sl. rancid	12.0
6	.36	rancid	13.2	rancid	17.3
10	.39	v. rancid	21.5	v. rancid	23.4
14	1.05	rancid, yeasty	42.0	v. rancid	32.6

TABLE XIX

Comparative Lipolytic Action of O. lactis in Cream and in Butter

Days held at	Cream			Butter	
	Per cent acidity	Flavor	Acid number	Flavor	Acid number
5° C.					
0	0.12	good	0.5	good	0.5
4	.21	old	1.5	good	.9
6	.23	old	5.7	old	2.3
10	.28	rancid	13.4	sl. old	3.6
14	.37	v. rancid	15.6	rancid	4.1
15° C.					
0	.12	good	.5	good	.5
4	.23	old	5.3	good	1.3
6	.29	old	8.3	sl. old	2.5
10	.42	rancid	15.3	sl. rancid	12.4
14	.57	v. rancid	19.4	rancid	14.8
21° C.					
0	.12	good	.5	good	.5
4	.32	rancid	8.2	old	11.0
6	.47	rancid	17.5	old	14.3
10	.68	v. rancid	31.3	v. rancid	33.4
14	1.05	v. rancid	43.0	v. rancid	38.5

greater at 5° than at 15° and 21° C. It is recognized that the amount of working butter receives influences the rate of growth of the organisms it contains. Since it was impossible to accurately control the degree of working, comparisons between the rate of fat breakdown in the various lots of butter containing different organisms should not be seriously considered. However since all lots of cream were very similar in fat content and other properties it seems logical that comparisons could be made of the lipolytic activity in cream of the various organisms studied.

All of the lots of experimental cream and butter became off-flavored and unmarketable after a few days of storage at all temperatures studied. Using the flavor of the butter and the acid number of the fat as criteria, it may be concluded that all of the organisms studied were extremely damaging to the quality of cream and butter. It may be further concluded that the bacterial species studied, including Ach. lipolyticum, Ps. fluorescens and Alc. lipolyticus (data on latter two not shown), were less damaging to cream and butter from the standpoint of fatty decomposition than either O. lactis or Myc. lipolytica.

Effect of Neutralization of Sour Cream on the Acid Number of the Fat

Raw cream was inoculated with a culture of a lipolytic organism, Ach. lipolyticum, and incubated until the acidity of the cream and the acid number of the fat had increased appreciably. To a series of quart jars, each containing 1 pound of cream at 30° C., was added neutralizer in increasing amounts so that portions of cream at different acidities were obtained. Sodium carbonate and magnesium oxide were used. The cream was then pasteurized at 62° C. for 30 minutes and cooled. The lots of cream were churned and acid numbers of the fat were determined.

While the data (table XX) reveal a very definite reduction of the free acidity in the fat, the neutralization of this acidity was somewhat slower and less complete than the neutralization of the serum acidity. The free fatty acids were reduced in all samples of cream to which alkali was added but the rate of reduction was slow until the titratable acidity of the cream was reduced appreciably. Using sodium carbonate, comparatively little reduction was noted in the acidity of the fat until the titratable acidity of the cream had been reduced to 0.20 per cent; with magnesium oxide, to 0.15 per cent. After sufficient alkali had been added to reduce the cream to the neutral point using phenolphthalein as an indicator, some free acid still remained in the fat. Although there was no appreciable difference in the degree of reduction of the fatty acids by the two alkalies, magnesium

TABLE XX

Effect of Neutralization of Sour Cream on Acid Number of the Fat

Cream pasteurized at 62° C. for 30 minutes

Treatment of cream	Neutralized with Sodium carbonate: Per cent acidity in cream	Sodium carbonate: Acid number of fat	Magnesium oxide: Per cent acidity in cream	Magnesium oxide: Acid number of fat
Raw	0.72	2.1	0.72	2.1
Pasteurized, not neutralized	.71	1.9	.71	1.9
Pasteurized and neutralized	.40	1.8	.44	1.8
	.29	1.8	.33	1.8
	.20	1.4	.27	1.7
	.17	1.0	.15	1.2
	.13	.8	.11	1.2
	.10	.7	.10	1.2
	.00	.4	.00	.2

oxide appeared to be slightly more effective than sodium carbonate. Results of experiments by Bird and Breazeale (3) show that a definite reduction in the fatty acids of cream occurred when it was neutralized.

The work of Clark, et al. (8) included a study of the acid numbers of many samples of commercial butter of varying quality. They considered that a good correlation existed between the acid numbers of the fat of butter and the quality of the cream from which it was made and concluded that on the average, the poorer the quality the cream the higher the acid number of the fat of the resulting butter.

In general, the lots of high quality butter had low acid numbers on the fat. It did not follow however that low quality cream always produced butter with the higher acid numbers on the fat. Poor quality butter made from neutralized cream, often showed a relatively low acid number, depending on the degree of fat hydrolysis, the type and amount of neutralizer used in the cream. Judging from these data and from the results of others (3), one is not justified in assuming that butter with a low acid number on the fat was made from good quality cream.

A study was made on cream delivered to an Oklahoma Cooperative creamery, as related to the effect of the neutralization process on the acid number of the butterfat. In each trial the cream was received at the creamery, weighed, sampled and dumped into the pasteurizing vat without regard to quality. When the vat was full, the cream was warmed to about 30° C., a sample of the mixed cream was removed from the vat and immediately cooled. The acidity of the cream in the vat was then standardized to

approximately 0.25 per cent with a lime neutralizer. After the cream had been pasteurized and cooled a second sample was removed from the vat. A sample of butter was also taken from the completed churning. The samples of cream were churned and the acid numbers of the fat of the cream as well as of the butter were determined.

The titratable acidities of the unneutralized cream (table XXI) varied from 0.47 to 0.82 per cent and the acid numbers of the fat from 1.2 to 5.9. After standardization of the cream with alkali to acidities ranging from 0.23 to 0.27 per cent, the acid numbers of the fat ranged from 1.0 to 1.5. The acid numbers of the fat of the resulting butter ranged from 0.9 to 1.7. In every instance the acid number of the fat was reduced when the acidity of the cream was standardized. While there was considerable variation in the acid numbers of the fat of the unneutralized cream, the acid numbers after neutralization were comparatively uniform. This indicates that the percentage reduction of the fat acidity due to neutralization was considerably greater in the samples of raw cream with high acid numbers than in the samples with low acid numbers. The cream with the high acid numbers before neutralization made slightly lower quality butter than the cream with low acid numbers. Samples 8, 9 and 10 had higher acid numbers than the other samples and the resulting butter was of slightly lower quality.

TABLE XXI

Effect of Neutralization of Sour Cream on the Acid Number of the Fat of the Resulting Cream and Butter

All lots of cream from the mixed receipts of an Oklahoma Cooperative Creamery

	Churning number									
	1	2	3	4	5	6	7	8	9	10
Unneutralized raw cream										
Flavor	sour	sour	sour	sour	sour	sour	sour	sour, bitter	sour; sl. rancid	sour, off-flavor
Per cent acidity	0.68	0.64	0.82	0.65	0.69	0.72	0.47	0.70	0.75	0.69
Acid number of fat	1.5	1.9	2.5	1.5	1.5	1.2	1.4	4.1	5.9	5.7
Neutralized pasteurized cream										
Flavor	good	good	good	good	good	good	good	sl. bitter	old	old, off
Per cent acidity	.25	.26	.25	.27	.24	.26	.25	.24	.27	.25
Acid number of fat	1.0	1.4	1.5	1.4	1.0	1.1	1.2	1.3	1.4	1.2
Butter										
Flavor	coarse	coarse	coarse	acidy	old	coarse	clean, coarse	off-flavor	old	coarse
Score	90	90.5	90	89.5	90	90	91	89.5	89.5	89.5
Acid number of fat	.9	.9	1.0	1.0	1.0	.9	1.0	1.7	1.4	1.0

SECTION III

RELATION OF VOLATILE ACIDITY OF BUTTERFAT TO RANCIDITY

Effect of the Growth of Various Lipolytic Microorganisms on the Percentages of Total Acid of Fat that are Volatile and Non-volatile

During a study of many cases of rancidity in experimental butter caused by the growth of microorganisms, an excellent opportunity was afforded to obtain information concerning the relationship between the volatile and non-volatile acidity in the fat of rancid butter. Portions of sterilized sweet cream were inoculated with the organisms to be studied. The cream was then incubated at 15° or 21° C. for 6 days. The acid numbers and the volatile acidities of the fat were determined in the usual manner and the percentages of total acid that were volatile were calculated; the percentages of the total acid that were non-volatile were obtained by difference. The four trials with each organism were purposely not run simultaneously and because of the fact that cultures of different ages were used and different incubation temperatures employed, the degree of fat hydrolysis in the trials was not uniform. The object was to determine whether the organisms would hydrolyze the fat into the same volatile-non-volatile acid relationship under varying conditions of growth.

The average percentage of the acid that was volatile in the four trials with O. lactis was 1.9 (table XXII). In other trials not reported, slightly higher values were obtained but none of them were over 5.0 per cent. With Mye. lipolytica the percentage of total acid that was volatile was higher

TABLE XXII

Volatile and Non-Volatile Acidity Relationships in Butterfat Produced by the Growth of Pure Cultures of Lipolytic Microorganisms in Cream

The volatile acidity values represent the milliliters of N/10 sodium hydroxide required to neutralize the acid in 200 ml. of distillate when 10 gm. of fat were steam distilled

Sterilized cream inoculated and held 6 days

Trial	Temperature of incubation	Acid Number of fat	Volatile acidity	Per cent of total acid in the fat	
				Volatile	Non-volatile
O. lactis					
1	13° C.	18.6	0.45	2.4	97.6
2	13°	23.8	.5	2.1	97.9
3	21°	32.8	.6	1.8	98.2
4	21°	26.4	.4	1.5	98.5
Average:	---	----	---	1.9	
Ps. lipolytica					
1	13°	9.9	.9	9.1	90.9
2	13°	12.0	1.0	8.3	91.7
3	21°	21.0	1.7	8.1	91.9
4	21°	16.8	1.4	8.3	91.7
Average:	---	----	---	8.4	
Ps. fluorescens					
1	13°	3.7	.7	18.9	81.1
2	13°	5.8	.8	13.8	86.2
3	21°	7.6	1.0	13.1	86.9
4	21°	6.5	.85	13.1	86.9
Average:	---	---	----	14.7	
Ach. lipolyticum					
1	13°	2.8	.35	12.5	87.5
2	13°	4.7	.5	10.6	89.4
3	21°	7.1	.8	11.5	88.5
4	21°	8.3	.95	11.4	88.6
Average:	---	---	---	11.5	
Alc. lipolyticus					
1	13°	5.4	.65	12.0	88.0
2	13°	8.7	1.0	11.5	88.5
3	21°	14.9	1.6	10.7	89.3
4	21°	12.8	1.4	10.9	89.1
Average:	---	----	---	11.2	

than with O. lactis, the average being 8.4. All the species of bacteria studied gave relatively high volatile acid values and were uniform in their volatile-non-volatile acid relationship. Four trials each with Ps. fluorescens, Ach. lipolyticum, and Alc. lipolyticus gave averages of 14.7, 11.6 and 11.2 per cent, respectively.

There was a relatively close relationship between the volatile and non-volatile acid values in all the trials with an organism, regardless of age of culture used for inoculating the cream, incubation temperature or degree of hydrolysis as shown by the acid numbers. The different organisms varied considerably in the degree of hydrolysis produced, as well as in the percentages of the total acid that were volatile and non-volatile. The O. lactis culture, as has previously been shown, was actively lipolytic but the volatile acidity of the fat on which it had acted was somewhat low as compared with all other organisms studied. This observation confirms the belief of Orla-Jensen (28) who suggested that the organism utilized in its metabolism, the volatile fatty acids liberated by its growth.

For reasons previously cited the degree of hydrolysis caused by the same organism in different trials, as determined by acid numbers, varied somewhat. With a few exceptions, all the trials with an organism gave volatile acid values that were quite uniform, regardless of the degree of hydrolysis. In the case of O. lactis the acid numbers ranged from 18.6 to 32.8 but the volatile acid percentages varied only from 1.5 to 2.4. With Myc. lipolytica the acid numbers ranged from 9.9 to 21.0 and the

volatile acid percentages from 8.1 to 9.1. With Ach. lipolyticum the acid numbers varied from 2.8 to 8.3 and the volatile acid percentages from 10.6 to 12.5; other bacterial species produced volatile acid values similar to those produced by Ach. lipolyticum. The organisms studied varied considerably in the relative percentages of the total acid liberated that were volatile and non-volatile. In all trials with the same organism however this relationship was comparatively uniform.

One organism may have attacked the fat of cream in a somewhat different manner than another, or at least the end products of the metabolic processes were different. With O. lactis only a relatively small percentage of the total acid was volatile after the mold had grown while with all bacterial species studied a comparatively large percentage remained after growth. With all the bacterial species studied about the same percentages of the total acid produced from the fat were volatile which in all cases were considerably higher than those produced by either Myc. lipolytica or O. lactis.

Volatile and Non-volatile Acidity Relationships in the Fat of Commercial Butter Showing Rancidity

In the trials reported in other sections of this thesis the acid numbers of the fat of many samples of fresh cream were determined. Without exception the acid numbers of fresh fat were less than 1.0 and were usually between 0.5 and 0.6. Volatile acidity determinations on these samples of fat invariably yielded such low values that no dependence could be placed on them because they were usually lower than the limit of error of the titration method employed.

Among the many samples of commercial butter examined there were a number which were rancid. Acid number and volatile acidity determinations were made on the fat of these samples.

The data shown in table XXIII reveal that the samples of commercial butter which were described as rancid in some degree had acid numbers on the fat ranging from 1.3 to 14.0. Samples 1, 4 and 5 were described as rancid with acid numbers of 4.8, 5.6, and 5.8, respectively, while samples 3 and 9 were described as slightly rancid and had acid numbers of 10.8 and 14.0, respectively. Sample 13 was very rancid and had an acid number of only 1.6 and sample 14 was rancid with an acid number of only 1.3. There was no correlation between the intensity of the rancid flavor and the acid number of the fat.

Samples 1 to 12, inclusive, revealed relatively little variation in

TABLE XXIII

Volatile and Non-Volatile Acid Relationships in the Fat of Commercial Butter Showing Rancidity

Sample	Flavor	Acid number	Volatile acidity*	Per cent of total acid in the fat	
				Volatile	Non-volatile
1	rancid	4.8	0.8	16.7	83.3
2	very rancid	6.2	1.0	16.1	83.9
3	sl. rancid	10.8	1.55	14.3	85.7
4	rancid	5.6	.65	11.6	88.4
5	rancid	5.8	.95	16.4	83.6
6	sl. rancid	2.8	.45	16.1	83.9
7	sl. rancid	3.2	.45	14.1	85.9
8	rancid	2.4	.4	16.7	83.3
9	sl. rancid	14.0	1.9	13.6	86.4
10	rancid	4.8	.55	11.4	88.6
11	rancid	7.6	1.15	15.1	84.9
12	sl. rancid	5.2	.75	14.4	85.6
13	very rancid	1.6	**	----	----
14	rancid	1.3	**	----	----

* See table XXII.

**Quantity too small to measure accurately.

the volatile-non-volatile acid relationships of the fat acidity. The percentages of the total acid that were volatile ranged from 11.4 to 16.7. Samples 13 and 14 had very low acid numbers and did not yield a sufficient quantity of volatile acidity to measure accurately by the method employed.

In another section of this thesis it was shown that little or no correlation existed between rancidity and acid number of the butterfat. In the data (table XXIII), it may be noted that the acid number of the fat and the percentage of the total acid that was volatile was not related directly to the intensity of the rancid odor. While it is rather unsatisfactory to determine the degree of rancidity by organoleptic tests, no other method exists which will detect the defect with equal reliability. Probably the agent responsible for the hydrolysis is an important factor in determining the degree of rancidity that will accompany a certain acid number on the fat. For example, in some experimental trials, samples of butterfat on which O. lactis had acted showed relatively high acid numbers with no indication of rancid odor. It is possible, as has been suggested by Orla-Jensen (28), that certain lipolytic molds are able to consume the volatile acids as rapidly as they are liberated from the fat yielding a fat with a relatively high acid number and yet showing no signs of rancidity.

Hammer (17), Hunziker (19) and others have stated that the odor of rancid butter is due to the presence of some of the lower fatty acids particularly, butyric, caproic and caprylic. Grossfeld and Battay (14) reported that one part of butyric acid in 12,500 parts of a medium could be detected by sense of smell. Stark and Scheib (35) believed that amounts

of butyric acid in rancid butter may be so small that though they can be detected in butter by tasing and smelling, they cannot be measured by ordinary chemical means. Since the acid numbers of some of the samples of good butter, as shown in table I, were very high and the acid numbers of some of the rancid samples shown in table XXIII were low the agencies responsible for rancidity must have exerted a selective action on certain of the glycerides of the fatty acids. Only the higher acids must have accumulated in the good butter showing a high acid number on the fat. Conversely, in the rancid samples having very low acid numbers, a relatively large percentage of the total acid accumulated must have been volatile. From the data presented it is evident that no definite relationship existed between the quantity of acid liberated from the fat and the degree of rancidity present.

The relationships between the volatile and non-volatile acidities of the fat of samples 1 to 12 of the rancid butter were comparatively uniform regardless of the acid numbers of the fat which ranged from 2.4 to 14.0. There was no relationship between the degree of rancidity and the volatile acidity, that is, in general the slightly rancid samples had the same volatile-non-volatile acid relationship as did the rancid and very rancid samples.

In conclusion, the percentages of the total acid in the fat that were volatile varied only slightly in the samples of rancid butter studied. There was no correlation between the percentages of the total acid in the fat that were volatile and the degree of rancidity.

Ability of Various Lipolytic Organisms to Utilise Salts of the Lower Fatty Acids as the Sole Source of Carbon

Both experimental and commercial rancid butter showed some variation in the volatile-non-volatile acid relationships of the fat. Various workers have suggested that certain microorganisms utilize some of the lower volatile fatty acids in their growth. In order to determine whether the organisms used in the previously reported experiments could grow in media in which a sodium or calcium salt of a single volatile fatty acid comprised the sole source of carbon, a series of such media were prepared following the general formula of Ayres, et al. (1). These media had the following composition:

sodium ammonium phosphate	2.0 gm.
potassium chloride	.1 gm.
salt of fatty acid	5.0 gm.
distilled water	1000 ml.

The salts used were sodium and calcium butyrate, calcium caproate and calcium caprylate. Forty ml. portions of each medium were placed in glass containers with screw caps and sterilized in the autoclave. The media were then inoculated with microorganisms to be studied and incubated at 21° C. After 7 days and also after 14 days of incubation, a complete series of the inoculated media were treated as follows:

The contents of each bottle were placed in a Kjeldahl flask containing

225 ml. of distilled water. Five ml. of N/1 sulphuric acid were added to each flask to free any remaining fatty acid from the salt. The flasks were then placed on the distilling apparatus and heated until 200 ml. of distillate were obtained. These distillates were titrated against N/10 sodium hydroxide, using phenolphthalein as an indicator. Handled in an identical manner, uninoculated 40 ml. portions of each medium served as checks. It was assumed for comparative purposes that any decrease in the volatile acid obtained from a medium after growth of an organism, compared with the check, was due to utilisation of the acid by the growing organisms.

There was no apparent uniformity in the ability of different organisms to utilise the fatty acids (table XXIV). O. lactis grew luxuriantly in the media containing sodium and calcium butyrate and lowered the volatile acid obtainable from the sodium butyrate medium from 16.1 ml. (check) to 3.4 ml. after 7 days and to 1.2 ml. after 14 days. Almost complete disappearance of the butyric acid may be noted. Similar reductions were shown in the calcium butyrate and calcium caprylate media. In the calcium caprate medium some growth was evident but it was not nearly so luxuriant as in the other media. The data further substantiate earlier suggestions that O. lactis is able to utilise volatile fatty acids.

Myc. lipolytica grew in all the media but grew less luxuriantly than O. lactis in the medium containing calcium butyrate, as determined by the titration values after 14 days, the value for O. lactis being 0.6 ml. and for Myc. lipolytica 11.0 ml. This organism showed greater growth after 14 days in the media containing the calcium salts of caproic and caprylic

TABLE XXIV

Ability of Certain Lipolytic Microorganisms to Utilize the Salts of the Lower Fatty Acids as the Sole Source of Carbon

The values represent the milliliters of N/10 sodium hydroxide required to neutralize the acid in 40 ml. of medium. The difference between the values given for an organism and the check sample (no inoculation) on the same medium represents the milliliters of N/10 acid utilized by the growing organism

Organism	Sodium butyrate		Calcium butyrate		Calcium caproate		Calcium caprylate	
	Days of incubation at 21° C.							
	7	14	7	14	7	14	7	14
O. lactis	3.4	1.2	3.7	0.6	6.9	6.8	1.1	0.9
Myc. lipolytica	12.1	5.0	11.3	11.0	5.2	4.8	1.3	.3
Ps. fluorescens	12.5	12.3	13.0	13.0	4.7	3.6	2.0	1.7
Ach. lipolyticum	16.2	16.4	17.0	17.1	7.2	7.2	2.2	1.9
Alc. lipolyticus	12.5	9.4	10.2	8.3	7.2	7.2	2.2	1.5
Check (no inoculation)	16.1	16.2	17.5	17.1	7.2	7.2	2.9	2.9

acids than did O. lactis. Ps. fluoreseens utilized all of the salts to some extent; Ach. lipolyticum did not show appreciable growth in any of the media and consequently utilized very little of the fatty acids. Alc. lipolyticus utilized sodium and calcium butyrate but was unable to utilize the calcium salts of caproic and caprylic acids to any extent.

The organisms studied varied greatly in ability to use the salts of the lower fatty acids as their sole source of carbon. The fact was established, however, that some of the organisms studied were definitely able to destroy by their growth certain of the volatile fatty acids. Coolhaas (10) showed that certain bacteria were able to ferment a large number of fatty acid salts. It is possible also that certain organisms may be able to act on the higher fatty acids in such a manner as to split off acetic acid causing increased titration values. It is therefore evident that the acid number of a fat is not an exact index of the degree of hydrolysis of the fat. Another point to consider is that even though certain organisms utilized the lower fatty acids in synthetic media in which the fatty acids were the only source of carbon, this does not necessarily prove that they would utilize them when growing in cream or butter. Under different circumstances they might obtain their carbon from a more readily available source and leave the fats unhydrolyzed. These results establish the possiblity of the utilization of the lower fatty acids by certain microorganisms growing in cream.

SECTION IV

CHANGES IN THE ACID NUMBER OF BUTTERFAT DURING STORAGE

Effect of Storing Filtered Butterfat at Various Temperatures on the Acid Number of the Fat

During the course of the studies difficulty was encountered occasionally in titrating all the fat samples in a series at approximately the same time. Some samples filtered more slowly than others and in some cases it was necessary to leave samples of fat at 45° C. overnight and titrate them the next morning. In order to determine the effect of holding the filtered fat for varying periods on the acid number, 26 samples with a considerable variation in acid numbers were stored at 45° C. and the acid values determined originally, after 2 weeks and, with some of the samples, after 6 weeks. The samples were from several sources. Some were from commercial butter and others from miscellaneous experimental lots; a portion of them were rancid.

At the beginning of the trials the fat varied in acid numbers (table XIV) from 0.65 to 13.0. Without exception the acid numbers after 2 weeks storage were practically the same as initially. Twelve of the samples were held for 6 weeks and no appreciable changes in the acid numbers occurred although slight increases were observed with two samples - samples 20 and 26. Apparently the agencies responsible for hydrolytic decomposition were not active in pure fat at 45° C. during the 6 weeks storage period. In the two samples in which slight increases in acid numbers occurred tallowiness was noted but other samples showing no acid number

TABLE XXV

Effect of Storing Filtered Butterfat at 45° C. on the Acid Number of the Fat

Some of the samples from commercial butter and others from experimental butter

Sample	Acid number of fat		
	Before storage	After 2 weeks	After 6 weeks
1	0.8	0.8	
2	1.4	1.4	
3	.65	.65	
4	2.65	2.65	
5	3.95	3.95*	
6	3.95	4.0	
7	2.2	2.2	
8	2.35	2.3	
9	3.05	3.1	
10	2.5	2.6*	
11	3.25	3.25	
12	3.65	3.7	
13	7.9	7.9	
14	5.3	5.2	
15	1.0	1.05	1.05*
16	1.25	1.2	1.15
17	.7	.75	.75
18	3.4	3.4	3.4
19	5.85	5.8	5.8*
20	6.5	6.5*	6.75*
21	2.0	2.1	2.1
22	2.5	2.4	2.3
23	1.4	1.4	1.5
24	6.85	6.8	6.75
25	11.7	11.6*	11.6*
26	13.0	13.0*	13.5*

* Samples tallowy.

increases also became tallowy during storage.

In order to compare the changes in the acid number of filtered fat at various temperatures, samples of fat were stored for 6 weeks at 5°, 13°, 21°, 37° and 45° C.; the acid numbers were determined at intervals of 1 week.

The data (table XXVI) reveal that the rancid fat in trial 1 did not change appreciably in acid number during 6 weeks at 5°, 13° or 21° C., while the non-rancid fat in trial 2 resisted changes at all five temperatures. The rancid samples stored at 37° and 45° C. began to show small increases in acid numbers after about 5 weeks. These results varied somewhat from those shown in table XXV, in that in these trials slightly greater increases in acid numbers were noted after 6 weeks at 45° C. The rancid samples held at 37° and 45° C. became tallowy after 3 weeks and 2 weeks, respectively. After tallowiness became evident the acid numbers increased slightly throughout the remainder of the storage period.

The acid number of filtered butterfat did not change appreciably during storage for 2 weeks at temperatures ranging from 5° to 45° C. Slight increases in acid numbers were noted in the rancid samples held at 37° and 45° C. after 5 and 4 weeks, respectively. None of the samples held at 21° C. or lower for 6 weeks showed any increase. While most of the samples observed did not show appreciable acid number increases even after 6 weeks, a few did, therefore it is not safe to assume that changes would not occur after periods exceeding 2 weeks at 45° C. The fat can be held without change for considerably longer periods at 21° C. and lower.

TABLE XXVI

Effect of Storing Filtered Butterfat at Various Temperatures on the Acid Number of the Fat

Trial 1
Acid number at the beginning 5.0; fat rancid

Temperature of storage	Acid number of butterfat after storage (weeks)					
	1	2	3	4	5	6
5° C.	5.0	5.0	5.0	5.1	5.1	5.1
13°	5.0	5.0	5.0	5.0	5.0	5.0
21°	5.0	4.9	5.0	5.0	5.0	5.0
37°	5.0	4.8	5.1*	5.1*	5.5*	5.6*
45°	5.1	5.1*	5.1*	5.3*	5.7*	6.2*

* Samples tallowy.

Trial 2
Acid number at the beginning 0.65; fat not rancid

Temperature of storage	Acid number of butterfat after storage (weeks)					
	1	2	3	4	5	6
5° C.	0.65	0.6	0.65	0.65	0.65	0.7
13°	.65	.65	.6	.7	.75	.7
21°	.7	.65	.7	.65	.65	.65
37°	.65	.7	.65	.65	.7	.7
45°	.65	.65	.65	.6	.65	.7

Effect of Lipolytic Microorganisms on the Acid Number When Inoculated into Filtered Fat and into a Fat-Water Emulsion

It is generally accepted that pure fat will not support growth of microorganisms. Schreiber (33) reported that fat alone was not a suitable nutrient medium for microorganisms, but that in the presence of other nutrients and oxygen certain organisms could destroy the fat. He found this process proceeded most rapidly in the presence of calcium carbonate and when the fat was in a finely divided state. In order to determine whether the method used in preparing the fat for acid number determinations removed the food elements other than fat sufficiently to prevent the growth of microorganisms, the following experiments were performed. Tubes containing 10 gm. of rancid fat and others containing the same quantity of non-rancid fat were inoculated with certain lipolytic organisms. To one series of the tubes sterile distilled water was added to the extent of 25 per cent of the volume of the fat, the tubes being shaken until some degree of emulsification was evident and the fat had solidified. Both series of tubes were then incubated at 21° C. and the acid numbers were determined after 14 and again after 30 days of storage.

In the trials using both the rancid and non-rancid fat (table XXVII) there were no appreciable changes in the acid numbers of the fat alone or of the fat in the fat-water emulsion. Growth of organisms in the pure fat

TABLE XXVII

Effect of Lipolytic Microorganisms on the Acid Number When Inoculated into Filtered Fat and into a Fat-Water Emulsion

Trial 1
Acid number at the beginning 5.6; fat rancid

Organism	Acid number of fat after storage at 21° C.			
	Pure fat		Fat-water emulsion	
	14 days	30 days	14 days	30 days
O. lactis	5.6	5.4	5.6	5.3
Myc. lipolytica	5.5	5.6	5.6	5.5
Ps. fluorescens	5.7	5.4	5.5	5.5
Ach. lipolyticum	5.6	5.4	5.4	5.3
Alc. lipolyticus	5.5	5.6	5.4	5.6
Check (no inoculation)	5.6	5.3	5.6	5.4

Trial 2
Acid number at the beginning 0.5; fat not rancid

Organism	Acid number of fat after storage at 21° C.			
	Pure fat		Fat-water emulsion	
	14 days	30 days	14 days	30 days
O. lactis	0.5	0.55	0.55	0.5
Myc. lipolytica	.55	.5	.5	.55
Ps. fluorescens	.5	.5	.5	.6
Ach. lipolyticum	.55	.6	.55	.55
Alc. lipolyticus	.6	.55	.55	.55
Check (no inoculation)	.55	.5	.6	.55

would not be expected even in the presence of suitable nutrient material because of the absence of moisture, but in the trials in which water was present it would be logical to expect some growth if the proper combination of nutrient materials was present, even in small quantities. Since no changes in the acid numbers were evident after 30 days storage it was assumed that no growth took place with any of the organisms studied and that the filtered fat was stable toward the action of lipolytic organisms.

These data support the conclusion that certain organisms which were definitely able to hydrolyze fat in cream and in butter were unable to hydrolyze filtered butterfat or the fat in a butterfat-water emulsion. These results indicate that any changes in the acid number of butterfat during a 30 day storage period at 21° C. were probably due to agencies other than microorganisms.

DISCUSSION OF RESULTS

After subjecting good quality, unsalted commercial butter from many sources to keeping quality tests, rancidity was a common defect. All of the samples were made from pasteurized cream. Since lipase is readily inactivated or destroyed by heat, and since there was little chance for contamination of the cream with the enzyme after pasteurization, the rancidity which developed probably was the result of the growth of microorganisms. Most of the lipolytic organisms commonly found in milk and cream are readily destroyed by ordinary pasteurization temperatures, therefor the organisms responsible must have gained entrance subsequent to pasteurization. Very few of the samples of salted butter observed became rancid when subjected to keeping quality tests. The salt evidently was very inhibitory to the organisms responsible for the rancidity, since the opportunities for contamination of salted butter were essentially the same as with the unsalted butter.

The acid numbers of the fat of the fresh, unsalted samples were uniformly low, usually less than 1.0. After holding at 21° C. for 6 days, many of the samples showed increased acid numbers. However, there was no definite correlation between the acid numbers of the fat and the quality of the butter after storage. While high acid numbers usually accompanied the development of rancidity, rancid samples with low acid numbers were sometimes encountered. Conversely, samples not showing rancidity,

frequently had relatively high acid numbers. In the rancid samples with low acid numbers on the fat, the proportions of the total fat acid that were volatile, while often unmeasurable by the method employed, probably were considerably higher than in the rancid samples with relatively high acid numbers.

Lipolytic organisms varied considerably in their ability to produce rancidity in unsalted butter. Some organisms which definitely caused hydrolysis of fat, as evidenced by increased acid numbers, failed to cause a rancid condition. O. lactis, for example, greatly increased the acid numbers of the fat in cream or in butter and yet, in some instances, a rancid flavor did not develop. Three bacterial species produced rancidity regularly even with very slight increases in the fat acid values. Differences in the proportions of total acid that were volatile as a result of the growth of the various organisms were significant and accounted for the conditions mentioned.

Lactic acid in cream in amounts greater than are normally present, either produced by the growth of the common lactic organisms or added directly, had no effect on the acid number of the fat. The acid was completely absent from the fat after churning, apparently being left in the buttermilk. Acid tended to inhibit the growth of the lipolytic organisms but was not effective in controlling them. Ripening cream to a relatively high acidity for unsalted butter no doubt aids in the control of certain organisms. This practise, however, cannot be expected to prevent growth of the undesirable types.

The growth of organisms in unsalted butter was somewhat more limited than in cream. In butter, the food supply was not as plentiful as in cream and because of the physical structure of butter the organisms were somewhat confined and were less able to migrate to new food supplies. The organisms studied, however, were all very detrimental to the quality of unsalted butter stored at temperatures as low as 5° C.

The neutralizing process, as applied to sour cream in the manufacture of butter, was definitely effective in lowering the acid number of the fat. This indicates that the alkali not only neutralized the water soluble fatty acids that were in the serum but also partially neutralized the acids present in the fat. It is probable that the hydrolysis of fat in cream is essentially a surface phenomenon, since the lipase is water soluble and is present chiefly in the serum. The possibility that only the surfaces of the fat globules are acted upon during the neutralization process offers an explanation for the fact that even though the titratable acidity of cream was reduced lower than the phenolphthalein end-point, the fat still had a positive acid value. The fact that the fatty acids in the cream fat are largely neutralized during the processing of the cream preparatory to churning eliminates the possibility of a good correlation between the acid number of the fat of butter and the quality of the cream from which it was made.

In trials in which O. lactis was grown in cream or butter, the percentage of the total acid liberated that was volatile was very small. Certain organisms, particularly O. lactis, were capable of growing in a medium in which salts of the lower fatty acids provided the sole source of

carbon. It is probable that O. lactis largely consumed the volatile acids liberated from the fat. However, it is possible that this organism may have exerted a selective action on the fat, liberating only the higher acids, and that it would not have consumed the lower fatty acids in the synthetic media if other food materials had been available. With other organisms grown in cream or butter, the ratios of volatile to non-volatile acids liberated were very uniform in all trials with the same organism, which indicated that the fat hydrolysis proceeded in a definite manner with each organism. This relationship prevailed in all trials with an organism regardless of varying growth conditions or the degree of fat hydrolysis produced.

In commercial unsalted butter showing rancidity this same uniform volatile non-volatile acid relationship was very evident, regardless of the degree of rancidity. In all of these samples the percentages of the total acids in the fat that were volatile were comparatively high. This might indicate that bacteria were chiefly responsible for the rancid condition in commercial unsalted butter, since O. lactis and Myc. lipolytica in pure cultures both produced relatively low volatile acid values.

Filtered butterfat from commercial unsalted butter was very stable toward hydrolytic changes when stored at temperatures ranging from 5° to 45° C. Butterfat alone was not a suitable food for microorganisms, for even when partially emulsified with water and inoculated with lipolytic organisms, no acid number increases resulted after 30 days at 21° C.

CONCLUSIONS

1. Most samples of unsalted butter increased in acid numbers of the fat during holding for 6 days at 21° C.
2. When samples of commercial unsalted butter were held at 21° C., approximately 25 per cent became rancid within 6 days.
3. No close correlation existed between the acid number of the fat and the quality of commercial unsalted butter; butter of good quality often had relatively high acid numbers, while some rancid samples had relatively low acid numbers.
4. When samples of commercial salted butter were held at 21° C., comparatively few of the samples became rancid in 6 days.
5. Of the two biological agencies causing fat hydrolysis in raw cream, organisms were found to be of greater significance than lipase.
6. In raw cream containing no formaldehyde, in which both lipase and microorganisms were active, the lipolysis was greater at 5° than at 15° or 21° C.; in cream containing formaldehyde, in which lipase only was active, the degree of hydrolysis increased as the holding temperature of the cream increased within the range studied.
7. The growth of butter culture organisms or L. bulgaricus in sterilized cream, resulting in titratable acidities ranging up to 0.89 per cent with the former and up to 2.02 per cent with the latter, failed to cause changes in the acid numbers of the fat.

8. The addition of lactic acid to sterilized cream in amounts sufficient to increase the titratable acidity up to 2.61 per cent did not cause changes in the acid numbers of the fat after holding 6 days at 21° C.

9. O. lactis, Myc. lipolytica and Ach. lipolyticum were definitely inhibited by the addition to cream of excessive amounts of lactic acid. However, they all grew well in cream containing sufficient added lactic acid to give a titratable acidity of approximately 1.0 per cent. The first two species caused lipolysis in cream with an acidity of 2.08 per cent.

10. O. lactis and all of the species of bacteria studied were inhibited somewhat by the growth of butter culture organisms in cream; Myc. lipolytica showed increased growth in the presence of the butter culture organisms. Lipolysis, even in high acid cream, was extensive enough with all organisms investigated to be of importance in cream quality.

11. With the exception of Ps. fragi, all organisms studied which showed lipolysis on agar plates containing fat caused increases in the acid numbers of the fat when inoculated into sterilized cream, although rancidity did not result in every instance.

12. All organisms studied were more actively lipolytic in cream than in butter, especially at 5° C.

13. When the titratable acidity of sour cream was reduced by the addition of an alkali, the acid number of the fat was also reduced, but not proportionately.

14. Because of the decrease in the acid number of fat resulting from the neutralisation process, it cannot be assumed that butter with a low acid number on the fat was made from good quality cream.

15. The different organisms studied varied considerably in the percentages of the total fat acid that were volatile and non-volatile. The average percentage of the total acid of the fat that was volatile in the trials with O. lactis was 1.9; with Myc. lipolytica 8.4; with Ps. fluorescens 14.7; with Aeh. lipolyticum 11.5 and with Alc. lipolyticus 11.2.

16. There was a relatively close relationship between the volatile and non-volatile acid values on the fat in all the trials with each organism, regardless of the age of the culture used for inoculating the cream, the incubation temperature or the degree of fat hydrolysis.

17. In samples of commercial unsalted butter showing widely varying degrees of rancidity, the percentages of the total acid in the fat that were volatile varied only slightly; there was no close correlation between the percentages of the total acid in the fat that were volatile and the degree of rancidity.

18. Certain lipolytic organisms grew well in media in which a sodium or calcium salt of butyric, caproic or caprylic acid was the sole source of carbon; others grew little or not at all in these media.

19. O. lactis grew more luxuriantly in all of the synthetic media than any of the other organisms investigated.

20. In general, filtered fat from commercial unsalted butter was very resistant to hydrolytic changes when stored at 5°, 13°, 21°, 37° or

45° C. No changes were noted in the acid numbers of any of the samples until after 2 weeks at 45° C.; many samples showed no changes at 45° C. even after 6 weeks.

21. Neither filtered fat nor a fat-water emulsion supported growth of any of the lipolytic organisms studied.

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