EFFECTS OF SOME MICROORGANISMS ON THE KEEPING QUALITIES OF UNSALTED AND SALTED BUTTER AS INFLUENCED BY THE DEGREE OF WORKING

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INTRODUCTION

When optimum growth conditions prevail for any microorganism its rate of reproduction is maximum. Its reproduction rate can be retarded by limiting food nutrients, removing moisture, changing temperature, altering the oxygen supply, changing the hydrogen-ion concentration, varying the amount of light and by adding preservatives. The rate may be lowered considerably whenever one or more of the most effective of these changes are brought about. Therefore increases in the number of organisms can be controlled to a certain extent when it is not practical to destroy the organisms.

Butter will not lend itself to any of the methods available for destroying microorganisms without sacrificing some of its original high quality. Therefore controlling the growth of organisms must be relied upon to improve the keeping quality of butter if presence of organisms is responsible for deterioration of butter while it is in storage.

Hammer (9) reports that contamination by the introduction of microorganisms after pasteurization frequently occurs in the commercial manufacture of butter even when great care is exercised to avoid it. In such cases, some of the benefits of pasteurization are nullified so far as the keeping quality of butter is concerned, and holding butter at fairly low temperatures is not sufficient to insure that the butter will retain its high quality over an extended period of time. Limiting the food nutrients upon which the organisms subsist would be one means of improving the keeping quality of butter in these instances. If the concentration of nutrients in a single moisture droplet in butter could be reduced sufficiently growth of any living organism in the droplet could be controlled. This is accomplished to a certain extent by dilution

of the buttermilk on the surfaces of butter granules with wash water, and imprisonment of most of the living organisms in these diluted buttermilk droplets. In this manner most of the organisms are separated from the larger portion of their food supply. Most of the food is contained in the smaller droplets which were incorporated in the butter granules during churning. It is thought by Rahn and Boysen (16) that there is no dilution of these smaller droplets of buttermilk. They (16) have calculated that more than 99.99 per cent of these would be sterile in properly worked butter if the total count of bacteria on the butter was 50,000 per gram.

It is possible to disperse added moisture so finely in butter that the number of droplets would be greater than the number of organisms commonly found in butter. This means that many droplets would contain only a single living cell. Not only is it possible that the diluted medium might hinder growth but the space within the small droplet would soon become so crowded that further reproduction would be practically impossible.

Salt retards the growth of most microorganisms. For this reason it has been used as a preservative for certain foods for many years. However when salt alone is depended upon to prevent food spoilage very high concentrations are necessary. Butter preserved in this manner certainly would not be marketable as a high quality food.

It is generally accepted that the small percentage of salt added to butter to increase its palatability is sufficient to improve its keeping quality over similar butter not salted. The added protection of limiting food nutrients by sufficient working offers a possibility of maximum keeping quality of salted butter when held at low temperatures

if lipolytic or proteolytic microorganisms have gained entrance to it.

Early investigations of the deterioration of butter were confined almost entirely to studies of intensity of flavor and odor defects and changes in titratable acidity. Some work was begun on determinations of substances formed from the decomposition of the natural constituents of butter, but these studies were very limited until recently. Most of these investigations dealt with fat acidity and pH.

There is need for more data concerning decomposition of fat in butter. Protein decomposition in butter also needs to be studied further because comparatively little work has been done relative to the amino nitrogen and ammonia content of butter showing protein decomposition. Little information is available concerning the abilities of various organisms to liberate amino nitrogen and ammonia when they cause a break down of proteins in butter.

The present study will be concerned with deterioration of butter as determined by flavor and odor defects, titratable acidity and pH of the butter plasma, acidity of the fat and the amino nitrogen and ammonia content of the plasma when known organisms are responsible for the deterioration. It will also be concerned with the most practical method for delaying or preventing deterioration for a reasonable time when microorganisms are responsible for lowering the quality of butter while in storage.

HISTORICAL

Butter, obtained by churning milk in crude churns of animal skins, was used for food by man even before recorded history. Few attempts were made by primitive man to preserve it to be used during seasons of the year when little or no milk was produced. The use of high concentrations of salt brine into which wrapped butter was submerged was the first attempt to prolong extreme deterioration, but even this frequently resulted in a product which was unedible according to our present standards. The preservation of many foods became very essential as more and more people were removed farther from the sources of food production. Because butter was very perishable at ordinary temperatures investigations were undertaken to find the causes for its deterioration and find methods to prevent it. This marks the beginning of studies to improve the keeping quality of butter during storage.

Holding butter at low temperatures was the greatest single factor in its preservation, but this did not entirely prevent deterioration. It was soon discovered that microorganisms were partially responsible for these changes during storage. Many of these organisms have been isolated and identified.

Because differences in flavors and odors of butter have the greatest influence on its score and market value most of the investigations concerning its deterioration have dealt with defects of these items. Claydon and Hammer (3) were able to detect the putrid defect in unsalted butter when a pure culture of Achromabacter putrefaciens was added to cream and the cream churned, and also when a similar culture was added to the water used for washing butter made from sterile cream. The rancid off flavor and odor which is according to Grimes (8) characteristic of the lower

fatty acids, especially butyric, was produced when certain lipolytic organisms caused hydrolysis of butterfat. O. lactis has been found to produce ketonic rancidity in butter. It is recognized by the fruity flavor and odor defect which resembles the aroma of apples and other fruits. Hammer (9) reported the isolation of an organism capable of producing a skunk-like odor in butter when inoculated into pasteurized cream and the cream churned. Numerous other organisms, some unidentified, that were used by Fouts (6) in studies on development of fat acidity, produced different flavor defects in butter. Stark and Scheib (18) studied 486 different organisms capable of splitting fat or digesting proteins.

In reviewing the literature on the effect of O. lactis, Wildman (19) states:

> "... a symposium of dairy scientists meeting in 1935 had failed to find O. lactis to be of importance in judging cream and butter."

After examining 570 samples of sour cream Wildman (19) was able to show a direct relationship between the percentage of samples having abnormal flavors and the amount of mold mycelia as determined by the M. B. B. method. Fouts (6) found that a rancid off flavor was produced and the acidity of the fat increased appreciably at the end of 14 days of storage at 5° C. when O. lactis was inoculated into sterile cream and the cream churned. Hunziker (11) lists Oidium lactis (Oospora lactis) as one of the better known microorganisms capable of hydrolyzing butterfat to a marked degree and producing a pronounced rancid flavor and odor.

Flake and Parfitt (4) investigating the numbers of organisms in salted butter made from sour cream observed a marked correlation between both high proteolytic counts and high lipolytic counts and poor keeping quality.

Commenting on the elimination of organisms from butter Hammer (9) states:

> "The complexity of the problem is emphasized by the instances in which a serious attempt is made to control all the sources from which the pasteurized cream and the butter can be contaminated only to find that certain churnings of butter develop cheesiness, rancidity or some other defect under commercial conditions."

Rahn and Boysen (16) found that overworked unsalted butter had better keeping qualities, as determined by titratable acidities, than underworked unsalted butter. They attribute this to an increase in the fineness of moisture dispersion in the overworked butter.

Long and Hammer (13) state:

> "With the finer dispersion of the moisture, the droplets that are infected do not contain as much food for the growth of organisms, and this results in less deterioration."

Reworking butter is harmful to its keeping qualities, according to Long and Hammer (14). These investigators found that reworking frequently increased growth rates of bacteria and commonly decreased the time required for the development of defects. Shephard and Olson (17) report that salt in butter retards the growth of microorganisms both at 32° F. and at 70° F.

The conclusion of Long and Hammer (13) was:

> "The measurement of some products formed in butter by organisms is probably a more satisfactory indication of their activities than plate counts."

Fouts (6) states:

> "Of the two biological agencies causing fat hydrolysis in raw cream, organisms were found to be of greater significance than lipase."

The relationship of fat acidity to butter quality has not received extensive investigation until very recently. Fouts (5) concluded that

no close correlation existed between the acidity of the fat and the quality of commercial unsalted butter. He reported finding a sample of commercial butter scoring 89.5 which had a fat acidity of 0.5 and another sample scoring 91.5 with a fat acidity of 1.7. In another investigation Fouts (7) found that there was no close relationship between the degree of rancidity and the percentage of total free fatty acids in the fat which were volatile.

Jacobsen (12), using Sorensen's method for determining the amino nitrogen in butter and expressing values as percentages of total nitrogen, found a general trend in which the amino nitrogen content increased as the quality of the butter decreased.

Comparisons of all deterioration factors in a single sample of butter are lacking. Also lacking is a comparison of the influence of the fineness of moisture dispersion in salted butter with a similar condition in unsalted butter.

STATEMENT OF PROBLEM

The work herein reported was undertaken to collect more complete data on the deterioration of butter and to obtain information concerning the effects of some microorganisms on the keeping qualities of unsalted and salted butter as influenced by the degree of working. In these studies consideration was given the following points:

I. A preliminary experiment to select some microorganisms commonly found in butter which are most harmful to its keeping qualities.

II. A comparison of Sorensen's and Van Slyke's methods for the determination of amino nitrogen in butter plasma from which the crude protein had not been completely removed.

III. The value of incubating samples of butter at 70° F. in predicting keeping qualities of similar samples of butter when stored at 40-45° F.

IV. The influence of the degree of working on the keeping qualities of unsalted butter and salted butter when certain organisms are present.

EXPERIMENTAL PROCEDURE

Equipment Used for Churning, Working and Preparing Samples

A churn for experimental purposes was made by fastening a special type 10 gallon cream can in a specially built frame which permitted end over end rotation similar to the hand type barrel churn. This churn was very desirable because it could be easily cleaned and sterilized. A rubber ring under the lid provided a seal. It was selected also because it was of the proper capacity for the amount of cream used in each churning.

Strainers made of cheese cloth were used to remove the coagulated lactalbumin from the autoclaved cream. Following churning and subsequent washing, each portion of butter was worked, using a small hand type wooden butter worker. Samples were placed into half pint milk bottles by means of slender wooden paddles. The bottles constituted the storage containers. In addition to the regular disc caps, parchment hoods held in place by rubber bands, were used to protect the samples from contamination.

Products Used

Each batch of cream used in this experiment was sweet cream from the college dairy herd. It was free from objectionable flavors and odors.

Sterile distilled water was used for washing the butter from each churning. Tap water was not used because its hardness varied considerably during such extended periods of time as used in this experiment.

A commercial grade of salt (sodium chloride), sterilized by dry heat, was worked into the salted samples. Attempts were made to incorporate 2 per cent salt into each portion of butter that was salted. This was considered to be the average amount in most commercial salted butter.

Microorganisms Used

The microorganisms used in this study were Achromobacter putrefaciens, Mycotorula lipolytica, Oospora lactis and Pseudomonas fragi.

Treatment of Apparatus

The churn was thoroughly washed by hand and steamed over a high pressure steam jet after each churning. As only one churning was made per week, the churn was washed again just before using. It was then subjected to prolonged steaming over the steam jet. Upon cooling, the churn was treated with a chlorine solution, 100 parts per million of available chlorine, as an added precaution against contamination of the cream from this source.

All storage containers, wooden paddles, strainers and the butter worker were autoclaved at 15 pounds pressure for 45 minutes to insure absolute sterilization.

Treatment of Products

Each batch of cream was standardized to 33 per cent butterfat. It was sterilized in the autoclave at 15 pounds pressure for 20 minutes. After allowing the cream to cool somewhat it was placed into a cold room maintained at about 40° F. where it was left for approximately 16 hours.

Distilled water, for washing the butter, was sealed in half gallon jars and sterilized in the autoclave. It was cooled to the desired temperature before using.

Preparation of Cultures of Microorganisms

After three consecutive transfers, two days apart, a culture incubated for 48 hours was used for the inoculation of each batch of cream. The mold, O. lactis, was grown on agar slants. The test tubes containing the growth covered slants were partially filled with sterile

water. Using a sterile inoculation needle, the mats of mycelia were broken up into very small particles which were distributed in the water. Four slants supplied the organisms used to inoculate one batch of cream. Pure cultures of Ach. putrefaciens, Myc. lipolytica and Ps. fragi were propagated in litmus milk. On the basis of 0.05 per cent inoculation, 10 ml. of a culture were used per batch of cream.

Churning and Working

All visible lactalbumin was removed from the autoclaved cream as it was strained into the churn. The cream was inoculated and churning started immediately. It was continued until the butter granules had increased to the size of small peas. After the buttermilk was drawn, the butter was washed twice with the sterile distilled water.

The butter from each churning was divided into six parts, three left unsalted and three salted. The unsalted portions were worked first.

One portion of the unsalted butter was worked slightly, at which point many droplets of moisture were visible on its surface. A second portion was worked a moderate amount after which only a few small droplets of water were visible on the surface of the butter. A third portion was sufficiently worked and was free of all visible moisture. The salted portions of butter were worked in a similar manner. Efforts were made to be consistent in the amount of working fiven all slightly, moderately and sufficiently worked portions of butter.

Taking Samples

All samples were transferred to the storage containers by means of sterile wooden paddles. Care was taken to avoid contamination.

After the desired degrees of working, samples from each of the six portions of butter were taken for each incubation period and each storage

period. Two additional sufficiently worked samples were taken, one unsalted and one salted.

Incubation and Storage

Samples of unsalted butter and salted butter representing the various degrees of working were incubated at 70° for 2, 4 and 6 days.

Other samples, taken from the same portions of butter as the samples for incubation, were stored at 40-45° F. for 4, 8, 12 and 16 weeks.

METHODS OF ANALYSIS

The original samples were examined for flavor and odor defects and analyzed by the various methods soon after they were taken. Each of the incubated samples was handled in a similar manner upon removal from the incubator. Samples removed from cold storage were allowed to become tempered at room temperature, about 30 minutes, before they were examined for flavor and odor defects. These samples were also analyzed by the various methods.

Flavor and Odor

The samples of butter were not scored. However, they were examined very critically for flavor and odor defects caused by the organisms used. The term "good" signifies that the only flavor criticism was the caramelized effect due to the heat treatment. "Off" means that the flavor and odor defect was not of sufficient intensity for unmistakable identification.

Preparing Samples

The samples of butter were warmed not to exceed 120° F. While in the liquid state they were poured into separatory funnels. These were placed in the 113° F. incubator in order to permit the fat and plasma to separate. Finally, the samples were centrifuged to bring about a more complete separation. The plasma was drawn off into test tubes. The fat was returned to the 113° F. incubator where it was filtered through paper into test tubes.

Acidity of Fat

A variation in the method devised by Breazeale and Bird (2) was employed to determine acidity of the fat. It consisted of weighing 10 gm. of filtered fat, into a 125 ml. Erlenmeyer flask. Ten ml. of

absolute alcohol and 25 ml. of petroleum ether were added and the mixture titrated with 0.1 normal alcoholic potassium hydroxide to the end point using alcoholic phenolphthalein as the indicator. The value derived in this manner is the acid degree of the fat and is expressed as the number of milliliters of 1 normal alkali required to neutralize the free fatty acids in 100 gm. of fat.

Amino Nitrogen Determinations

The milligrams of amino nitrogen were derived from values obtained by Sorensen's formal titration method and Van Slyke's micro-method; both are tentative (1). The values were obtained on samples of butter plasma from which no crude proteins were removed. Jacobsen (12) used Sorensen's method on cream and butter without removing proteins from these products. It is recognized that expressing milligrams of amino nitrogen from such results does not agree with values obtained by the methods outlined in A. O. A. C. (1). Nevertheless, the additional simplicity of the determinations and the smaller amounts of butter necessary for samples seemed to justify such an experiment on this product.

The first part of Sorensen's formal titration method for amino nitrogen, as modified by the author, involved titration of 10 gm. of plasma with 0.1 normal sodium hydroxide to the neutral point using phenolphthalein as the indicator. The determination was completed by adding 5 ml. of neutral formaldehyde (1) and subsequently titrating to the neutral point again with 0.1 normal sodium hydroxide. The milliliters of 0.1 normal sodium hydroxide used in the second titration minus the milliliters of the same base used in a blank titration times 2.8 equal the milligrams of amino nitrogen. This value is the same as is obtained by multiplying the milliliters of 0.2 normal base by 2.8 when a

20 gm. sample is used (1).

Whenever the term "amino nitrogen" is used to express the values obtained by Sorensen's method in this study it will be understood to include amino nitrogen, ammonia, and other substances in butter plasma which enter into a neutralization reaction with sodium hydroxide during the second titration of the determination as outlined previously.

In all determinations with Van Slyke's apparatus 1 ml. samples were used. Each sample was reacted upon exactly five minutes (stop watch) in the deaminizing bulb. Attempts were made to maintain the same rate of shaking for all determinations.

Room temperature and the barometric pressure were recorded as well as the milliliters of nitrogen gas for each determination. The milligrams of amino nitrogen were derived from the table prepared by Morrow (15). These were multiplied by 20 in order to have the values comparable to those by Sorensen's method.

Determination of pH

The Coleman glass electrode potentiometer was used for determining the pH of all butter plasma. Readings were made to the nearest 0.1 value and estimated with fair accuracy to the nearest 0.01 value.

Titratable Acidity

The titratable acidities were calculated from the milliliters of 0.1 normal sodium hydroxide used in the first part of Sorensen's method for amino nitrogen (see amino nitrogen determinations).

SECTION I

PRELIMINARY EXPERIMENT TO SELECT MICROORGANISMS

A few deviations from the outlined procedure were made in conduction of the preliminary experiment.

Cultures of 12 different organisms were used. Only sufficient sterile cream was inoculated and churned for one sample of unsalted butter for each organism. Churning was done in quart fruit jars. After the specified washing, each portion of butter was worked a moderate amount. This was done in small sterile pans by means of the sterile slender paddles.

The 12 samples of butter were incubated at 70° F. for 6 days. The methods of analyses were followed for all samples including one not inoculated which was analyzed soon after churning.

Results on 5 organisms are recorded in table I. All others produced smaller changes and are therefore omitted.

The bacterium, Ach. putrefaciens, was selected because it liberated amino nitrogen and produced the putrid defect. O. lactis, a mold, showed the greatest amount of lipolytic activity and imparted a fruity flavor and odor defect to the product. The yeast, Myc. lipolytica, produced the second highest fat acidity and some proteolysis. Fourth choice was Ps. fragi because of its ability to produce the rancid flavor and odor defect with very little lipolysis as shown by the small increase in fat acidity.

TABLE I

Deterioration of Butter by Microorganisms

(Cream Inoculated, Butter Churned and Held at 70° F. for 6 Days)

Quality determinations	Fresh butter	Changes produced by microorganisms				
		Ach. putrefaciens	Myc. lipolytica	O. lactis	Ps. fragi	Alc. lipolyticus
Flavor and odor	good	v. putrid	sl. putrid: sl. yeasty	v. fruity	rancid	----
pH of plasma	6.47	6.48	5.40	5.94	6.40	6.30
Per cent acidity in plasma	0.14	0.22	0.32	0.17	0.17	0.15
Milligrams of amino nitrogen in plasma (Van Slyke's method)	0.00	1.40	0.22	0.65	0.32	0.22
Milligrams of amino nitrogen in plasma (Sorensen's method)	4.48	7.28	6.16	2.80	5.32	4.34
Acidity of fat (acid degree)	1.95	1.95	8.70	13.00	2.20	2.15

Sl.-slight V.-very

SECTION II

COMPARISON OF SORENSEN'S AND VAN SLYKE'S METHODS OF DETERMINING AMINO NITROGEN IN PLASMA OF BUTTER

The milligrams of amino nitrogen in more than 300 samples of butter plasma were determined by both Sorensen's method and Van Slyke's method. The experiments involved studies on the effects of specific organisms on protein decomposition of unsalted butter and salted butter.

Table II contains data of 96 comparisons which are representative of all those obtained. These data show that Sorensen's method is of greater value than Van Slyke's method for determining protein decomposition in plasma of butter from which proteins were not completely removed.

The data obtained on Ach. putrefaciens show continual increases in amino nitrogen by Sorensen's method on all unsalted samples as storage periods were lengthened. These data also show an inconsistency in the values by Van Slyke's method on samples taken from the same portions of plasma. In one instance the value dropped from 4.14 to 0.96 in 4 weeks, and in another from 4.46 to 2.00 and then to 0.64 in consecutive 4-week intervals. The latter then rose to 4.86 in the last 4 weeks.

Data on the same organism show all values except one by Sorensen's method to be less in sufficiently worked than in slightly worked unsalted butter. Exactly the opposite was found by Van Slyke's method.

The data on Myc. lipolytica also support Sorensen's method. Slight irregularities were found in amino nitrogen values by Sorensen's method as storage periods were lengthened for samples of unsalted butter. However, data show very consistent decreases in amino nitrogen with increases in degree of working. Values by Van Slyke's method are also

Table II

Comparison of Amino Nitrogen in Butter Plasma as Determined by Sorensen's and Van Slyke's Methods

Weeks stored	Method of analysis	Milligrams of amino nitrogen					
		1	2	3	4	5	6
		Ach. putrefaciens					
4	Sorensen's	4.61	5.32	4.76	4.62	3.92	5.04
	Van Slyke's	4.14	3.48	4.46	3.90	2.60	2.94
8	Sorensen's	5.60	8.96	4.76	2.24	2.10	3.36
	Van Slyke's	0.96	3.86	2.00	2.34	----	0.22
12	Sorensen's	9.24	9.52	5.32	3.36	3.64	5.04
	Van Slyke's	1.38	1.92	0.64	0.96	0.96	----
16	Sorensen's	11.48	20.16	7.00	3.08	3.36	3.92
	Van Slyke's	2.64	16.30	4.86	1.40	11.64	1.48
		Myc. lipolytica					
4	Sorensen's	9.52	8.12	6.16	4.48	4.20	4.20
	Van Slyke's	4.64	3.54	0.32	1.21	3.10	1.98
8	Sorensen's	11.76	----	7.84	6.44	6.44	5.04
	Van Slyke's	5.70	3.28	2.40	1.10	0.32	----
12	Sorensen's	10.92	10.64	6.72	7.56	6.44	5.88
	Van Slyke's	4.58	2.66	1.80	1.28	1.92	1.16
16	Sorensen's	13.72	12.88	12.32	4.76	4.76	5.04
	Van Slyke's	5.72	9.26	5.92	1.98	2.80	0.84
		O. lactis					
4	Sorensen's	5.46	5.84	5.04	1.96	2.38	2.80
	Van Slyke's	0.64	2.14	1.38	0.10	0.74	1.06
8	Sorensen's	5.32	7.84	7.56	4.76	2.52	3.64
	Van Slyke's	0.52	1.26	1.26	2.00	1.06	1.26
12	Sorensen's	8.40	7.56	5.32	1.96	2.52	4.20
	Van Slyke's	2.24	2.32	0.96	----	0.74	----
16	Sorensen's	14.56	23.52	6.16	1.68	2.24	3.08
	Van Slyke's	7.28	17.14	3.22	2.08	0.09	0.07
		Ps. fragi					
4	Sorensen's	7.00	5.60	5.04	3.36	3.08	3.36
	Van Slyke's	----	----	----	1.52	0.30	0.64
8	Sorensen's	5.32	5.04	4.76	2.24	2.80	3.08
	Van Slyke's	0.84	1.90	----	----	0.10	0.20
12	Sorensen's	7.28	6.44	5.88	4.76	5.04	3.92
	Van Slyke's	1.70	1.60	2.24	1.38	2.98	2.34
16	Sorensen's	5.32	3.08	3.64	4.20	3.64	3.36
	Van Slyke's	1.16	4.32	0.42	0.20	1.26	----

1-Unsalted-----slightly worked-----4-Salted
2-Unsalted----moderately worked----5-Salted
3-Unsalted---sufficiently worked---6-Salted

fairly consistent in the latter comparison but fail to establish any correlation with storage time.

Data on O. lactis and Ps. fragi show trends on unsalted samples which tend to favor Sorensen's method slightly.

Very few of the comparisons of amino nitrogen on salted butter containing either of the organisms favor one or the other of the methods. Most values are so small and differences so insignificant that very little importance can be attached to them. The small advantages that were found favor Sorensen's method.

As the operation of Van Slyke's apparatus requires considerably more skill and time than are involved in determinations by Sorensen's method, the latter has the greater advantage if many determinations are to be made.

Perhaps Van Slyke's method could be used with an equal advantage to Sorensen's method on serum of dairy products. No data were obtained to establish any facts regarding this possibility.

It has been established that Sorensen's method excels Van Slyke's method for amino nitrogen on plasma of butter from which proteins have not been completely removed. Therefore no further use will be made of data by Van Slyke's method. Data by Sorensen's method will be used in all other amino nitrogen comparisons.

SECTION III

VALUE OF INCUBATING SAMPLES OF BUTTER AT 70° F. IN PREDICTING KEEPING QUALITIES OF THE SAME BUTTER STORED AT 40-45° F.

It was found (table III) that keeping qualities of sufficiently worked unsalted butter stored at 40-45° F. were predicted by values obtained on samples incubated at 70° F. for 6 days.

Flavor and odor defects and changes in amino nitrogen produced by Ach. putrefaciens at the end of 6 days at 70° F. corresponded very closely to values in a sample of the same butter stored 16 weeks at 40-45° F. The other values are not in close agreement. The small difference in the acidity of the fat can be attributed to experimental error.

In every instance, deterioration of unsalted butter caused by Myc. lipolytica was greater after 4 weeks at 40-45° F. than after 6 days at 70° F. By extrapolation, it was found that most values after 6 days at 70° F. compared with values after 2 weeks at 40-45° F. Predictions can be based on this correlation. These data show also that temperatures of 40-45° F. were not low enough to retard the growth of Myc. lipolytica appreciably.

Some inconsistency was found in the changes of values brought about by O. lactis on unsalted butter. By interpolation it was revealed that amino nitrogen and fat acidity values obtained after 6 days at 70° F. compared favorably with similar values found after 15 weeks at 40-45° F.

At 40-45° F. Ps. fragi grew nearly as luxuriantly as Myc. lipolytica. It produced the slight rancid defect in 4 weeks at 40-45° F. and the same defect but of greater intensity in 4 days at 70° F. In order to have comparable values it was necessary to interpolate those found on the samples stored at 40-45° F. The interpolated values were found to be

Table III

Comparison of Keeping Qualities of Sufficiently Worked Unsalted Butter when Incubated at 70° F. and when Stored at 40-45° F.

(Cream Inoculated with Specific Organisms)

Quality determinations	Days at 70° F.			Weeks at 40-45° F.			
	2	4	6	4	8	12	16
Ach. putrefaciens							
Flavor and odor	good	sl. putrid	sl. putrid	off	off	putrid	putrid
Per cent acidity in plasma	0.20	0.22	0.30	0.17	0.20	0.21	0.73
pH of plasma	6.39	6.39	6.22	6.51	6.50	6.50	4.98
Milligrams of amino nitrogen in plasma	5.60	6.30	7.14	4.76	4.76	5.32	7.00
Acidity of fat (acid degree)	2.05	2.05	2.05	1.92	----	2.00	2.10
Myc. lipolytica							
Flavor and odor	off	rancid	v. rancid	rancid	rancid	rancid	rancid
Per cent acidity in plasma	0.20	----	0.29	0.45	0.42	0.52	0.73
pH of plasma	5.99	----	5.60	5.38	5.02	4.92	4.90
Milligrams of amino nitrogen in plasma	4.90	----	5.32	6.16	7.84	6.72	12.32
Acidity of fat (acid degree)	1.90	----	4.85	7.40	11.90	15.70	20.60
O. lactis							
Flavor and odor	off	sl. fruity	sl. fruity	sl. fruity	sl. fruity	sl. fruity	fruity
Per cent acidity in plasma	0.15	0.30	0.22	0.20	0.36	0.26	0.32
pH of plasma	6.43	6.20	5.25	6.34	6.02	6.10	6.00
Milligrams of amino nitrogen in plasma	5.74	5.60	6.02	5.04	7.56	5.32	6.16
Acidity of fat (acid degree)	2.05	4.95	6.50	2.40	3.10	3.80	6.00
Ps. fragi							
Flavor and odor	off	rancid	rancid	sl. rancid	sl. rancid	sl. rancid	v. rancid
Per cent acidity in plasma	0.22	0.30	0.31	0.39	0.48	0.68	0.44
pH of plasma	6.12	5.87	5.82	5.54	5.40	5.19	5.12
Milligrams of amino nitrogen in plasma	6.16	5.04	5.04	5.04	4.76	5.88	3.64
Acidity of fat (acid degree)	2.60	3.75	4.60	5.30	7.80	9.70	12.00

Sl.-slight V.-very

about the same after 6 weeks at 40-45° F. as actual values after 6 days at 70° F.

Data show (table IV) that values obtained from samples of sufficiently worked salted butter incubated at 70° F. for 6 days are of little aid in predicting keeping qualities at 40-45° F. This is because changes in values as a rule are so small that they are within the range of experimental error.

By extrapolation of data on Myc. lipolytica it was found, however, that values after 6 days at 70° F. were about the same as values after 1½ weeks at 40-45° F.

Changes produced in salted butter by O. lactis after 6 days at 70° F. were about the same as those produced after 8 to 12 weeks at 40-45° F.

Table IV

Comparison of Keeping Qualities of Sufficiently Worked Salted Butter when Incubated at 70° F. and when Stored at 40-45° F.

(Cream Inoculated with Specific Organisms)

Quality determinations	Days at 70° F.			Weeks at 40-45° F.			
	2	4	6	4	8	12	16
	Ach. putrefaciens						
Flavor and odor	good	sl. putrid	sl. putrid	good	good	off	off
Per cent acidity in plasma	0.16	0.16	0.17	0.15	0.14	0.16	0.14
pH of plasma	6.00	6.02	6.04	6.06	6.00	6.07	6.03
Milligrams of amino nitrogen in plasma	5.32	4.48	5.04	5.04	3.36	5.04	3.92
Acidity of fat (acid degree)	2.00	2.00	2.05	1.92	----	2.00	2.00
	Myc. lipolytica						
Flavor and odor	good	good	off	sl. rancid	sl. rancid	sl. rancid	sl. rancid
Per cent acidity in plasma	0.16	0.16	0.18	0.24	0.32	0.38	0.46
pH of plasma	5.88	5.80	5.76	5.58	5.35	5.30	5.28
Milligrams of amino nitrogen in plasma	4.48	4.90	4.62	4.20	5.04	3.88	5.04
Acidity of fat (acid degree)	1.00	1.20	1.40	4.40	7.80	10.70	13.70
	O. lactis						
Flavor and odor	good	good	good	good	off	off	off
Per cent acidity in plasma	0.10	0.14	0.14	0.12	0.13	0.13	0.14
pH of plasma	5.95	6.02	5.92	5.92	5.97	5.95	6.01
Milligrams of amino nitrogen in plasma	3.50	3.92	3.64	2.80	3.64	4.20	3.08
Acidity of fat (acid degree)	1.85	1.95	2.00	1.90	1.90	2.00	2.10
	Ps. fragi						
Flavor and odor	good	off	sl. rancid	good	off	off	sl. rancid
Per cent acidity in plasma	0.12	0.12	0.17	0.17	0.15	0.19	0.14
pH of plasma	6.00	5.97	5.88	6.00	5.82	5.88	5.70
Milligrams of amino nitrogen in plasma	3.36	2.80	2.94	3.36	3.08	3.92	3.36
Acidity of fat (acid degree)	1.90	2.00	2.10	2.30	2.70	3.00	3.40

Sl.-slight V.-very

SECTION IV

INFLUENCE OF DEGREE OF WORKING ON KEEPING QUALITIES OF UNSALTED BUTTER AND SALTED BUTTER CHURNED FROM CREAM WHICH WAS INOCULATED WITH SPECIFIC ORGANISMS

Relation of Flavor and Odor of Stored Butter to Degree of Working

In most instances (table V) there were definite correlations between the development of flavor and odor defects in unsalted butter and the degree of working which the butter received. In order to eliminate repetition of the lengthy term "flavor and odor defect" the term "flavor defect" will be used to imply the same meaning.

During the first 4 weeks at 40-45° F. Ach. putrefaciens did not produce a flavor defect that could be identified. At the end of 8 weeks at the same temperature the putrid defect had developed in slightly worked butter and moderately worked butter. The sufficiently worked butter received the same flavor criticism given it at the end of the previous storage period. This butter developed the putrid defect after an additional storage period of 4 weeks.

The presence of molds, due to contamination, no doubt were responsible for the fruity flavor defects found in samples 1 and 2 after 12 and 16 weeks of storage. It is believed that the putrid defect would have been more pronounced had it not been masked in this manner.

Myc. lipolytica produced a very rancid flavor defect in slightly worked and moderately worked unsalted butter after 4 weeks storage. The same flavor defect but of lesser intensity was found in the sufficiently worked butter when it was examined after a similar storage period. The intensity of this flavor defect did not increase during the remainder of the storage periods. Fouts (6) found that this organism caused unsalted butter to exhibit a very rancid flavor defect at the end of 14 days of storage at 41° F.

O. lactis produced a slight fruity flavor defect in sufficiently

Table V

Effect of Degree of Working on Flavor and Odor of Unsalted Butter Stored at 40-45° F.

Degree of working	Flavor criticisms: Weeks in storage: Fresh	4	8	12	16
Ach. putrefaciens					
Slight	good	off	putrid	sl. fruity putrid	sl. fruity putrid
Moderate	good	off	putrid	fruity putrid	fruity putrid
Sufficient	good	off	off	putrid	putrid
Myc. lipolytica					
Slight	good	v. rancid	v. rancid	v. rancid	v. rancid
Moderate	good	v. rancid	v. rancid	v. rancid	v. rancid
Sufficient	good	rancid	rancid	rancid	rancid
O. lactis					
Slight	good	fruity	v. fruity	fruity	s. rancid fruity
Moderate	good	sl. fruity	fruity	sl. fruity	fruity
Sufficient	good	sl. fruity	sl. fruity	sl. fruity	sl. fruity
Ps. fragi					
Slight	good	rancid	rancid	v. rancid	v. rancid
Moderate	good	rancid	rancid	rancid	v. rancid
Sufficient	good	sl. rancid	rancid	sl. rancid	v. rancid

Sl.-slight V.-very

worked and moderately worked unsalted butter. This defect was more pronounced in butter which received only a slight degree of working.

Ps. fragi produced the rancid defect in all samples of unsalted butter after 4 weeks of storage. The intensity of this defect was less in the sufficiently worked than in the moderately or slightly worked samples.

The degree of working given salted butter (table VI) had a direct bearing on the development of flavor defects during storage at 40-45° F. Development of defects were deferred by sufficient working.

Ach. putrefaciens did not alter the flavor of sufficiently worked salted butter until the butter had been stored for 12 weeks. Even after 16 weeks storage the intensity of the defect was not sufficient for identification. This organism produced a slight tallowy defect in slightly worked salted butter during the first 4 weeks of storage. After 12 weeks a slight putrid defect had been produced.

The importance of sufficient working in retarding development of flavor defects is also emphasized by the differences produced by Ps. fragi. No change was found in flavor of sufficiently worked butter after 4 weeks of storage. Examination revealed moderately worked butter to be off in flavor and slightly worked butter to possess the slight rancid defect. The slight rancid defect was first detected in moderately worked and sufficiently worked butter after 16 weeks of storage. The intensity of this defect increased in the slightly worked butter during these same storage intervals.

After 4 weeks of storage there was no change in flavor of sufficiently worked salted butter made from cream inoculated with O. lactis. Even after 16 weeks no flavor defect which could be identified had

Table VI

Effect of Degree of Working on Flavor and Odor of Salted Butter Stored at 40-45° F.

Degree of working	Flavor criticisms: Weeks in storage: Fresh	4	8	12	16
Ach. putrefaciens					
Slight	good	sl. tallow	off	sl. putrid	sl. putrid
Moderate	good	sl. tallow	off	off	off
Sufficient	good	good	good	off	off
Myc. lipolytica					
Slight	good	rancid	rancid	rancid	rancid
Moderate	good	rancid	rancid	rancid	rancid
Sufficient	good	sl. rancid	sl. rancid	sl. rancid	sl. rancid
O. lactis					
Slight	good	off	off	off	sl. fruity
Moderate	good	off	off	off	off
Sufficient	good	good	off	off	off
Ps. fragi					
Slight	good	sl. rancid	sl. putrid	sl. rancid	rancid
Moderate	good	off	off	off	sl. rancid
Sufficient	good	good	off	off	sl. rancid

Sl.-slight V.-very

developed in this butter.

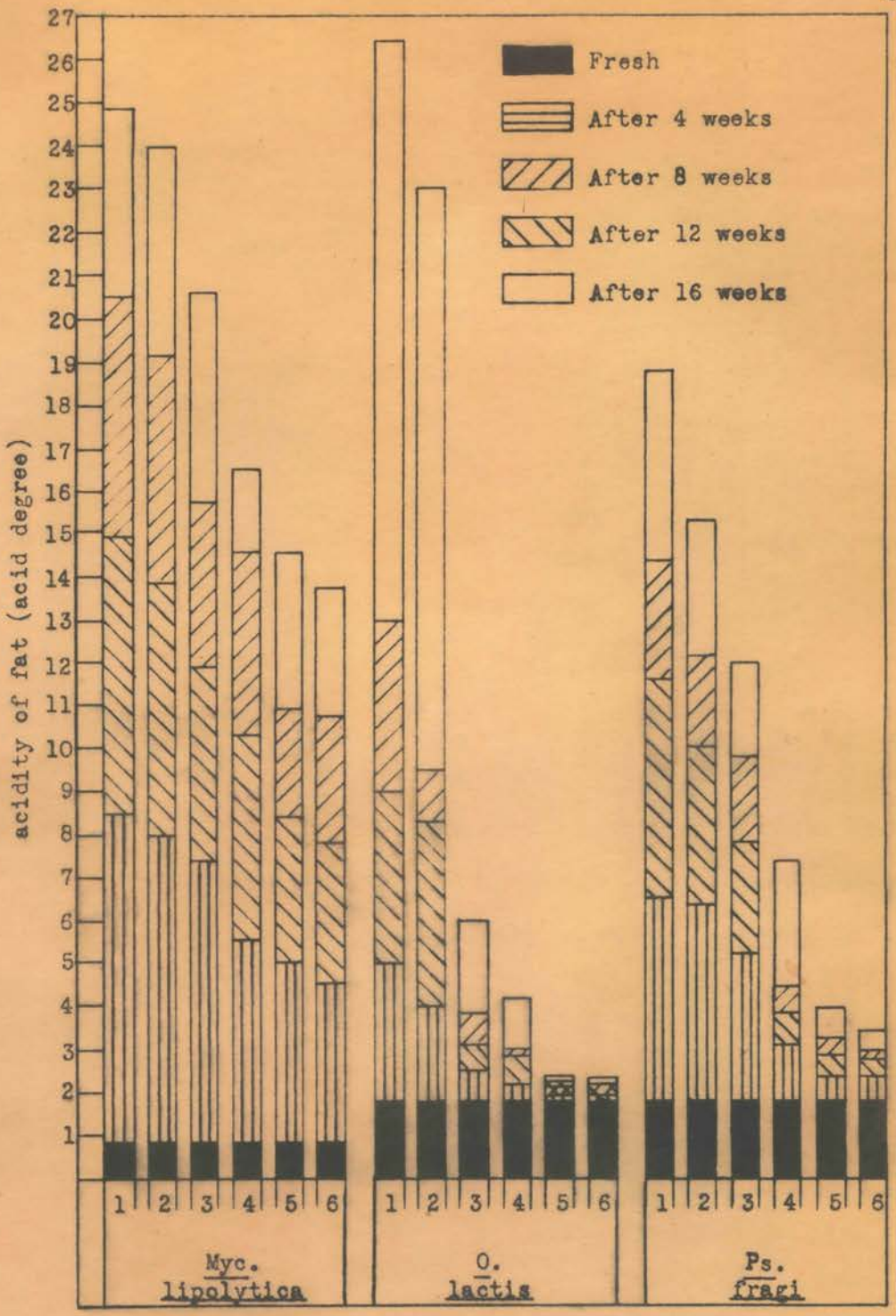
Relation of Acidity of Fat of Stored Butter to Degree of Working

Data show (figure 1) that the acidity of the fat was highest in slightly worked unsalted butter and lowest in sufficiently worked salted butter at the end of each storage period.

It was found that each organism produced lipolysis very rapidly in slightly worked unsalted butter at storage temperatures of 40-45° F. The damaging effect of each organism used was reduced somewhat in unsalted butter which was sufficiently worked. These effects were intermediate in unsalted butter which was moderately worked.

Sufficient working of unsalted butter that was stored at 40-45° F. was very effective in preventing large increases in fat acidity by O. lactis. This was true especially for 12 weeks of storage. Differences in fat acidity are not great enough to establish sufficient working as an effective means of controlling Myc. lipolytica and Ps. fragi.

The acidity of the fat was increased very little by O. lactis in sufficiently worked salted butter. The increase was almost negligible even after 16 weeks of storage. Sufficient working of salted butter was also effective in deferring lipolysis caused by Ps. fragi. Very little difference was found in the acidity of the fat of slightly worked and sufficiently worked salted butter when Myc. lipolytica was responsible for lipolysis.



1-Unsalted-----slightly worked-----4-Salted
2-Unsalted----moderately worked----5-Salted
3-Unsalted---sufficiently worked---6-Salted

Figure 1

Effect of Degree of Working on Acidity of Fat of Butter Stored at 40-45° F.

(Cream Inoculated with Specific Organisms)

Relation of Amino Nitrogen in Plasma of Stored Butter to Degree of Working

In general all organisms employed decomposed protein most rapidly in slightly worked unsalted butter.

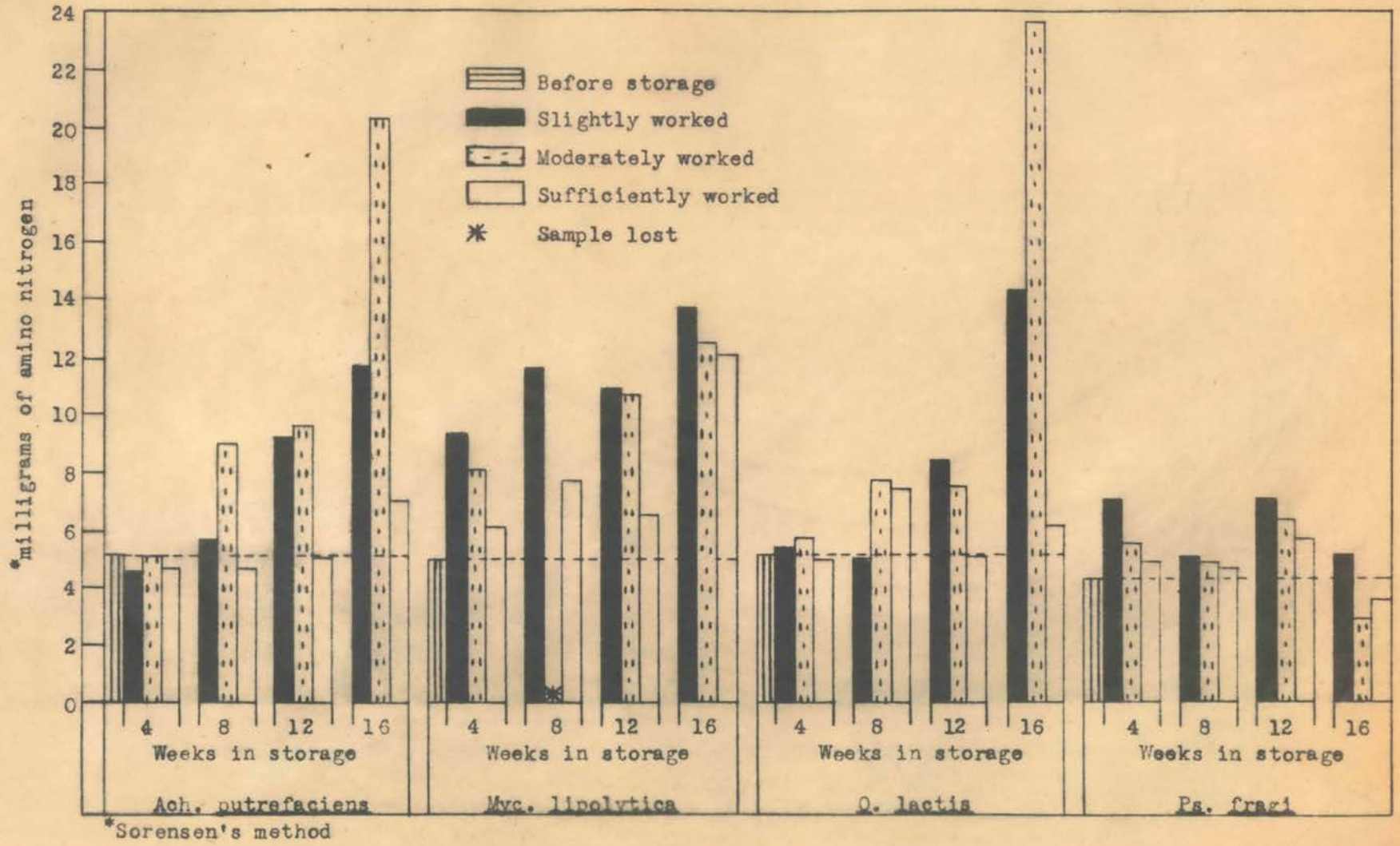
In a number of comparisons (figure 2) the milligrams of amino nitrogen were considerably less in the sufficiently worked butter than in the moderately worked butter. These differences were consistently great for all storage periods when proteolysis was caused by Ach. putrefaciens. Therefore these data support sufficient working as an effective means of controlling proteolysis in unsalted butter by this organism.

Data show that Myc. lipolytica caused more proteolysis in sufficiently worked unsalted butter than any other organism studied. This organism decomposed protein very rapidly in moderately worked butter as well as in slightly worked butter.

O. lactis liberated considerable amounts of amino nitrogen during 16 weeks of storage. The importance of sufficient working in controlling proteolysis caused by this organism is shown by the large differences in values obtained after 12 and 16 weeks. The same support was given sufficient working by the values obtained after each storage period when proteolysis was caused by Ps. fragi.

Protein decomposition in butter was controlled very effectively by the incorporation of 2 per cent salt.

Data show (figure 3) many of the amino nitrogen values to be less after storage than before storage. No explanation is offered for this condition. However it was noted that the milligrams of amino nitrogen were less in each fresh salted sample than in the corresponding fresh



*Sorensen's method

Figure 2

Effect of Degree of Working on Amino Nitrogen in Plasma of Unsalted Butter Stored at 40-45° F.

(Cream Inoculated with Specific Organisms)

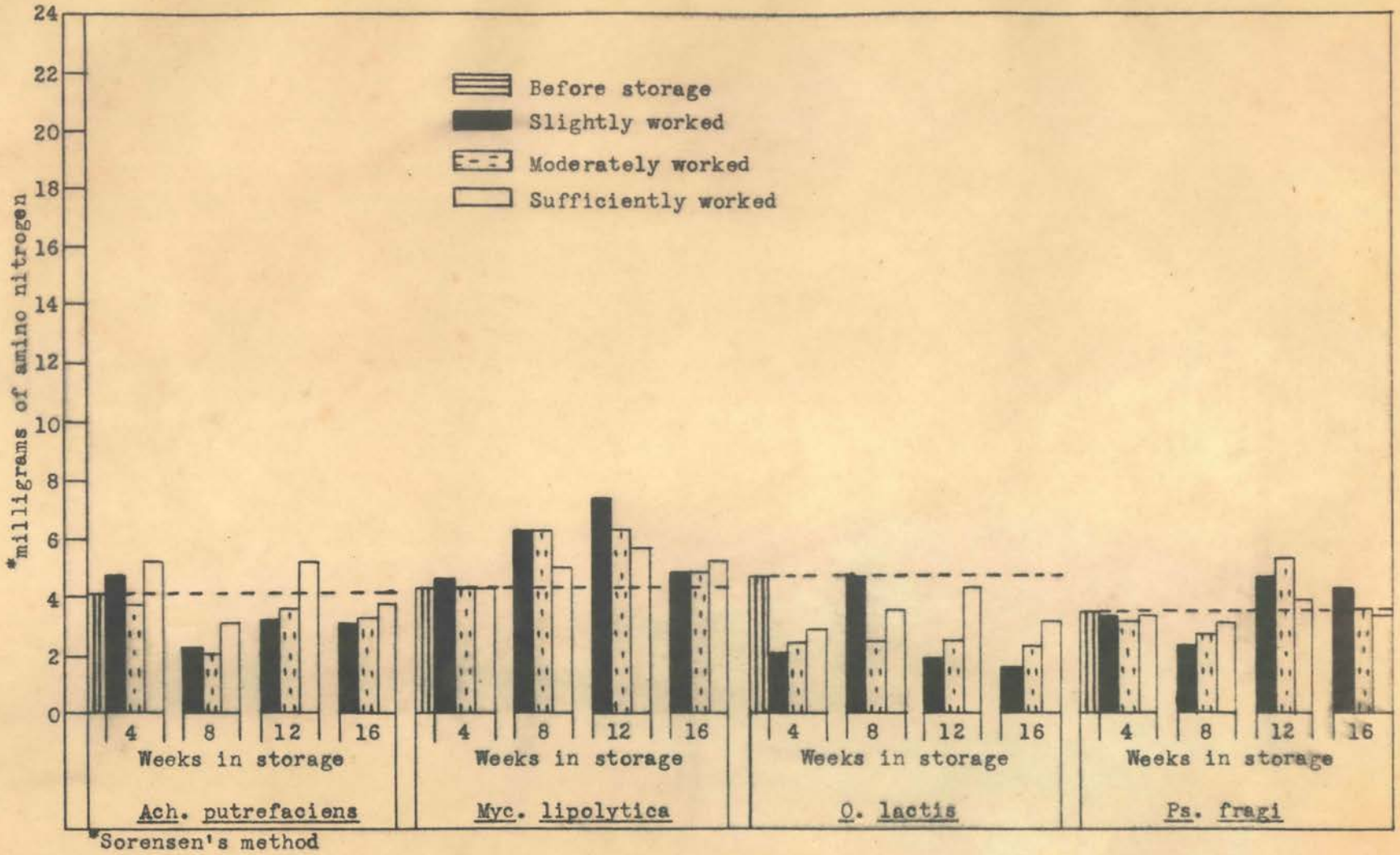


Figure 3

Effect of Degree of Working on Amino Nitrogen in Plasma of Salted Butter Stored at 40-45° F.

(Cream Inoculated with Specific Organisms)

unsalted sample.

The salt tolerance of Myc. lipolytica is indicated by its ability to liberate appreciable quantities of amino nitrogen in salted butter. Other organisms studied were able to do this only to a very limited extent.

Data on Myc. lipolytica and Ps. fragi support the statement that sufficient working is an aid in limiting protein decomposition in salted butter.

Relation of pH of Plasma of Stored Butter to Degree of Working

In general it was found that less change in pH had occurred in sufficiently worked than in moderately worked or slightly worked unsalted butter.

It is shown in figure 4 that all organisms reduced the pH of butter plasma as the periods of storage were increased. The smallest reductions were found with Ach. putrefaciens. The large decrease in pH of each sample after 16 weeks can be attributed to the mold which contaminated these samples. Myc. lipolytica reduced the pH of plasma of sufficiently worked unsalted butter far more during the first storage period than during any of the others. This organism caused differences in pH to become greater between sufficiently, moderately and slightly worked butter as storage was lengthened. Similar trends in pH were found with O. lactis and Ps. fragi. These data show the importance of sufficient working in controlling pH of plasma of unsalted butter.

Smaller changes in pH were produced by all organisms in salted butter than in unsalted butter. However sufficient working was an asset in controlling pH of salted butter as is shown in figure 5. The difference in pH between any two degrees of working with Myc. lipolytica and Ps. fragi are fairly consistent for each storage period.

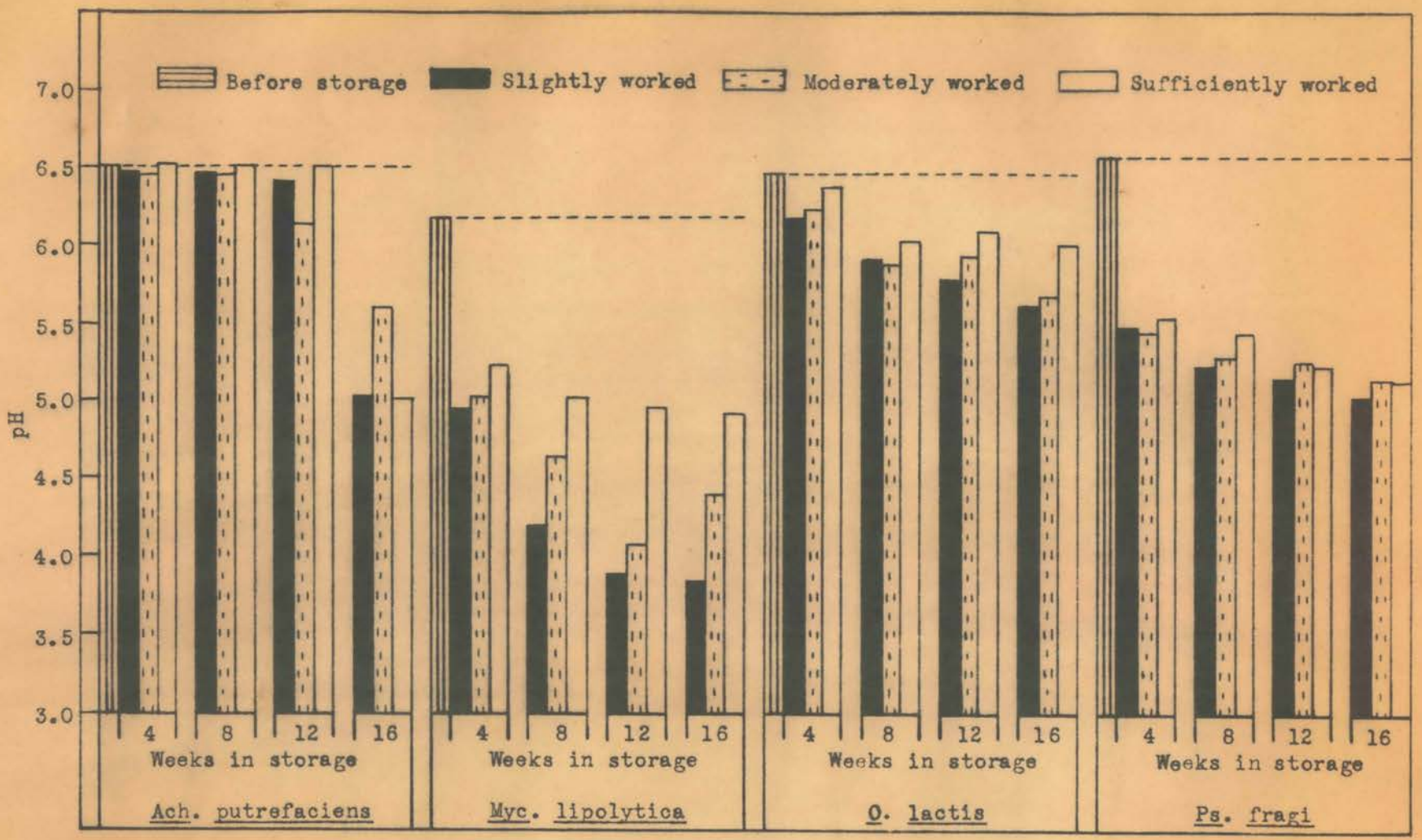


Figure 4

Effect of Degree of Working on pH of Plasma of Unsalted Butter Stored at 40-45° F.

(Cream Inoculated with Specific Organisms)

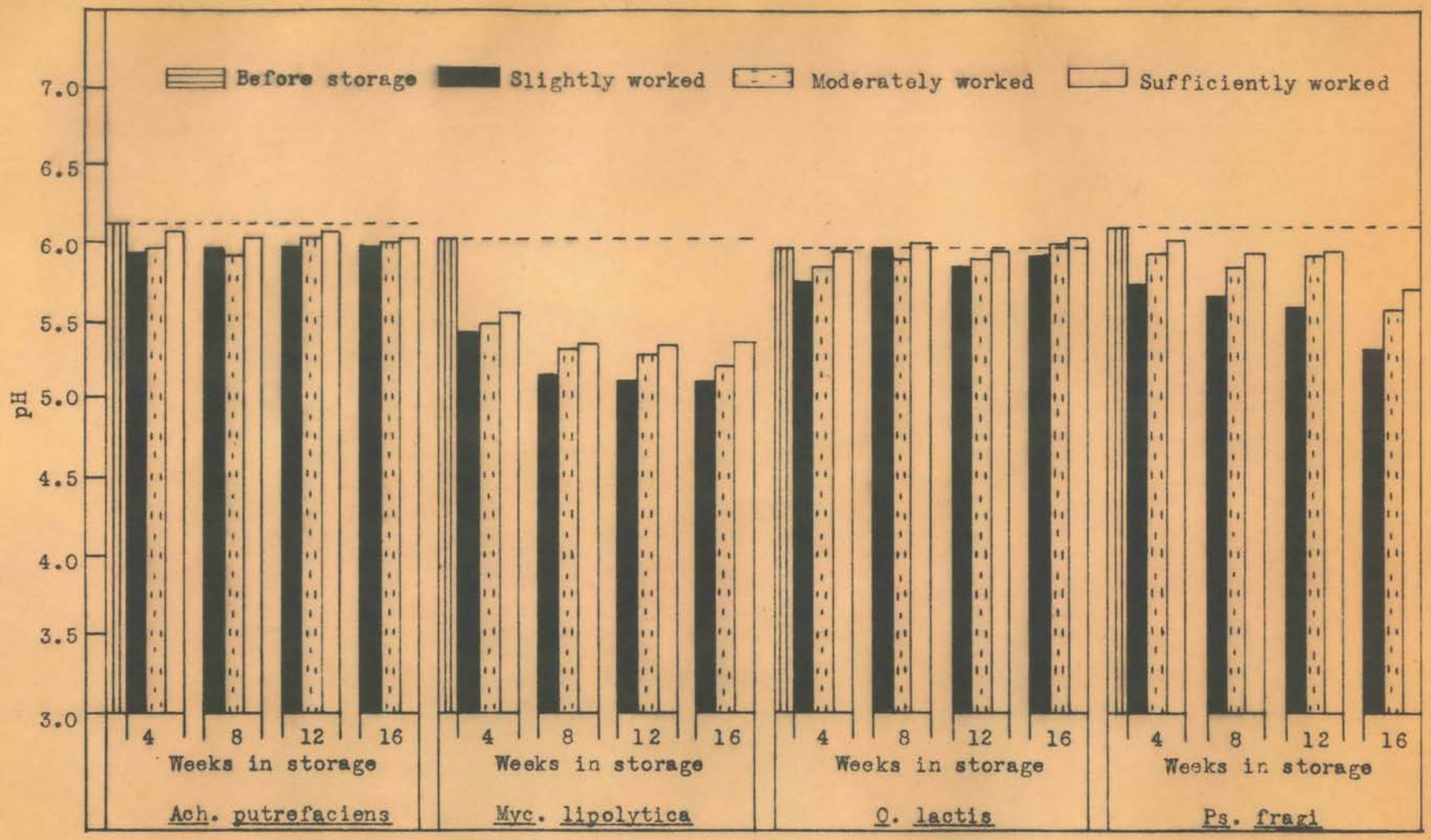


Figure 5

Effect of Degree of Working on pH of Plasma of Salted Butter Stored at 40-45° F.

(Cream Inoculated with Specific Organisms)

Relation of Titratable Acidity of Plasma of Stored Butter to Degree of Working

In general, the titratable acidity of the plasma of sufficiently worked unsalted butter was less than of moderately worked or of slightly worked unsalted butter (figure 6).

There was an increase in plasma acidity with increased storage regardless of the degree of working which unsalted butter received. The largest increase was found with Myc. lipolytica. This organism increased the plasma acidity considerably in four weeks as did Ps. fragi. Both increased plasma acidities nearly the same amounts in sufficiently worked butter with increased storage.

Data show that sufficient working was very important in controlling the plasma acidity in unsalted butter when Myc. lipolytica was responsible for changes in acidity. Also with O. lactis and Ps. fragi sufficient working was effective in controlling plasma acidity.

It is evident (figure 7) that Myc. lipolytica is more salt tolerant than any other organism studied. Ps. fragi was next in this respect.

An abnormal condition was found with Ach. putrefaciens and O. lactis. The titratable acidities of plasma of nearly all of these samples were less after storage than before storage. Because of the method of handling it is doubtful that any lactic acid had been formed in the cream prior to churning. Therefore these decreases likely could not be attributed to utilization of lactic acid by these organisms. The reduction in titratable acidity may be due to breakdown and utilization of the acid constituents of the plasma by the organisms. Most important of these are citric acid and phosphorus pentoxide.

Hammer (10) states that a high titratable acidity in salted butter

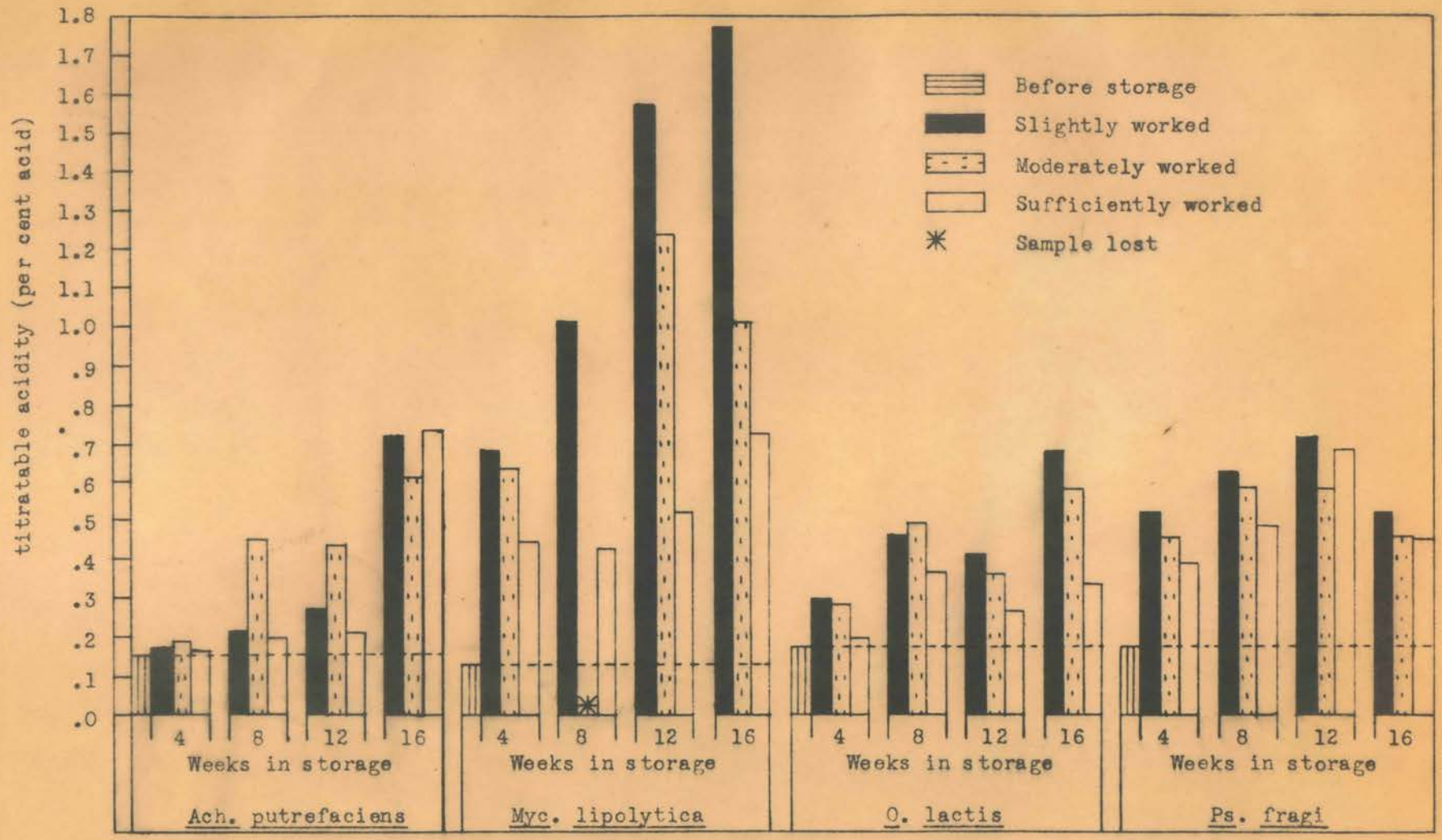


Figure 6

Effect of Degree of Working on Titratable Acidity of Plasma of Unsalted Butter Stored at 40-45° F.

(Cream Inoculated with Specific Organisms)

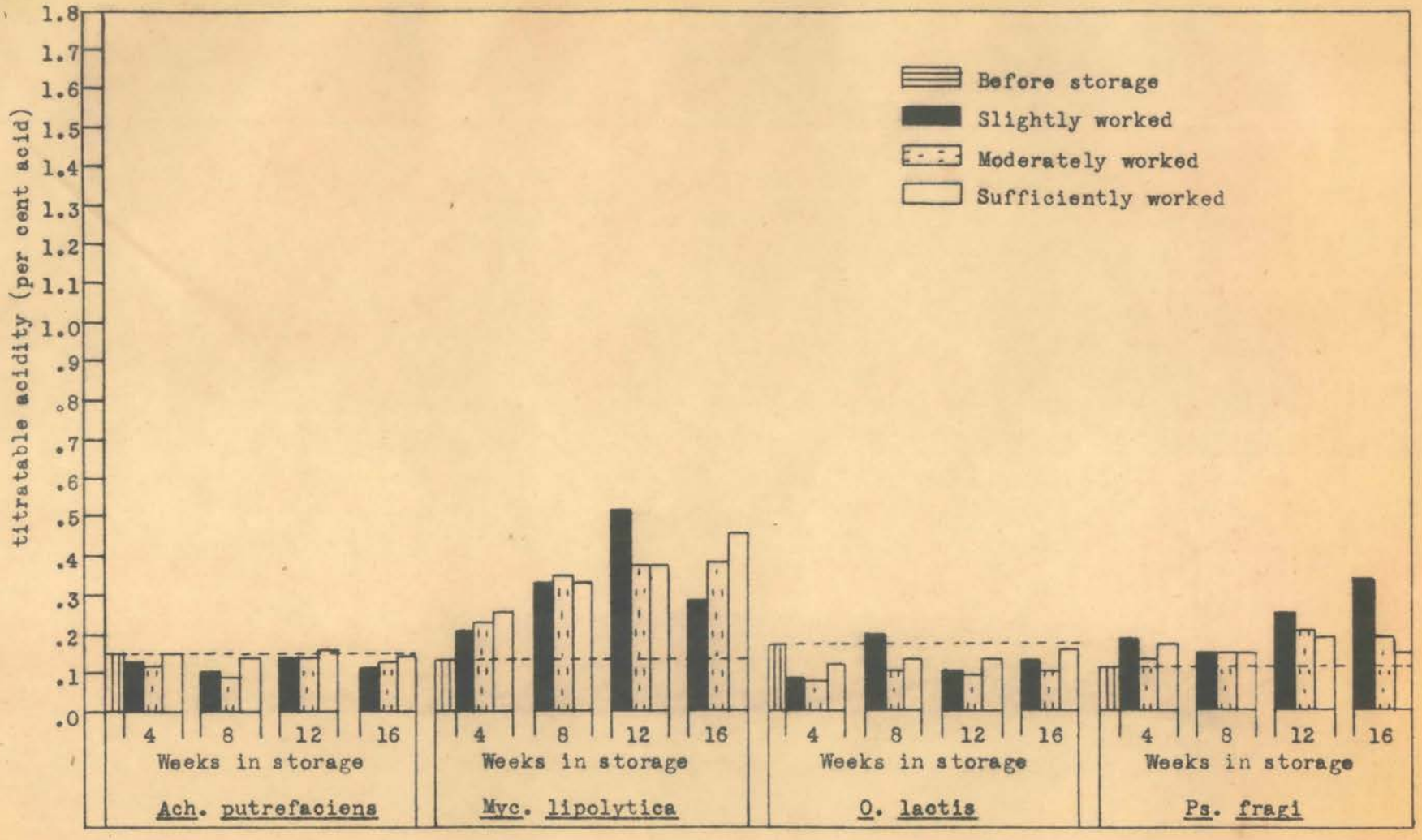


Figure 7

Effect of Degree of Working on Titratable Acidity of Plasma of Salted Butter Stored at 40-45° F.

(Cream Inoculated with Specific Organisms)

favors the development of a fishy flavor defect. It is therefore important that titratable acidities of salted butter be kept at a very minimum in commercial practices in order to assure good keeping quality of salted butter.

Sufficient working was important in preventing Ps. fragi from increasing titratable acidities of salted butter during storage. It was of little importance with Myc. lipolytica.

Table VII is introduced because it contains all data herein discussed except data in table I and the portions of tables III and IV dealing with incubation of samples at 70° F. Data for all figures were also taken from this table.

CONCLUSIONS

1. Sorensen's formal titration method was found to be more valuable than Van Slyke's micro-method for determining protein decomposition in plasma of unsalted butter. It was of little value, but more than Van Slyke's, on plasma of salted butter.

2. A 70° F. incubation period of 6 days was valuable in predicting keeping qualities of unsalted butter stored at 40-45° F. The value of the incubation period was almost negligible on salted butter.

3. Sufficient working was important in retarding the development of flavor defects in unsalted butter. It was of greater importance in controlling flavor defects in salted butter.

4. The acidity of the fat was highest in slightly worked unsalted butter and lowest in sufficiently worked salted butter.

5. Sufficient working was valuable in controlling protein decomposition in unsalted butter.

6. In most instances salt added to butter was more effective than sufficient working in preventing increases in amino nitrogen.

7. Smaller changes in pH occurred in sufficiently worked unsalted butter than in moderately or slightly worked unsalted butter. This was true also of salted butter.

8. Sufficient working was valuable in preventing appreciable increases in titratable acidity of plasma in unsalted butter.

9. Measurement of chemical changes in butter were found to be aids to the organoleptic method for determination of butter quality.

10. Sufficient working was valuable in controlling the growth of *Ach. putrefaciens*. Salt was also very effective.

11. Myc. lipolytica damaged butter quality to such an extent that the benefits of sufficient working and salt were not important.

12. The keeping quality of unsalted butter was severely damaged by O. lactis. Salt was very effective in reducing this damage.

13. Salt was more effective than sufficient working in controlling Ps. fragi.

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