

Bacteriology of Butter

VII. Effect of Reworking Butter on Growth of Bacteria

BY H. F. LONG AND B. W. HAMMER

AGRICULTURAL EXPERIMENT STATION
IOWA STATE COLLEGE OF AGRICULTURE
AND MECHANIC ARTS

DAIRY INDUSTRY SECTION

AMES, IOWA

SUMMARY AND CONCLUSIONS

1. With unsalted butter made from pasteurized cream inoculated with various organisms and held at approximately 10°C., reworking after several days had the following effects:

- a. The growth rates of bacteria frequently were increased.
- b. The time required for development of defects commonly was decreased.
- c. When butter culture was added to the cream, the pH values of the butter serum decreased more rapidly.
- d. When lipolytic organisms were added to the cream, the acidities of the fat increased more rapidly.

2. The printing of butter in a type of equipment which subjected it to reworking tended to aggregate the moisture.

3. The reworking of butter may accelerate various microbiological changes in the product. Distributing organisms to previously uninfected moisture droplets and providing a greater food supply for certain of the organisms in the butter by aggregating the moisture are apparently of importance in this connection.

Bacteriology of Butter

VII. Effect of Reworking Butter on Growth of Bacteria*

BY H. F. LONG AND B. W. HAMMER

The dispersion of the moisture in butter is an important factor influencing the growth of bacteria in this product. When a bacterial cell is in a large moisture droplet it has more food available than when it is in a small droplet and, if growth conditions are favorable, it can multiply more extensively and bring about more decomposition. Depending on the species involved, this greater decomposition can influence development of off flavors, decreases in pH, increases in acidity of the fat and other changes.

The wash water retained by butter and the water added to standardize the composition of butter should be distinguished from that coming from the cream. Presumably, the added water is more difficult to disperse in very small droplets, but on the other hand it contains less organic matter. Depending on various conditions, either type of water may be the more serious from the standpoint of harmful organisms.

The moisture dispersion in butter is influenced not only by the working during manufacture but also by the treatment the butter receives later. Occasionally, as a result of some irregularity, butter has a moisture content above the legal limit and is reworked to eliminate some of the water. During printing of butter with certain types of equipment, the general effect on the butter is comparable to reworking.

Various butter marketing organizations have reported that in some instances butter in prints has developed a defect due to the action of organisms, while butter from the same churning but marketed in tubs has not. Although this relationship suggests that the butter printer was a source of objectionable organisms, it commonly was in good sanitary condition and was not a cause of difficulty with the churning printed before or after the one showing conspicuous microbiological deterioration. The general situation indicates that the change in moisture distribution during the printing may have been a factor in the spoilage.

In order to study the effect of reworking butter on the action of bacteria in it, trials were conducted with various organisms, and the results are reported herein.

* Project 119 of the Iowa Agricultural Experiment Station.

THEORETICAL CONSIDERATIONS

Theoretically, reworking butter may have various effects on the activity of bacteria in it. If little bacterial growth has occurred in the butter and there is no tendency to aggregate moisture droplets during the reworking, there should be a finer dispersion of the moisture and a decreased food supply for the individual organisms, the effect being essentially the same as working at the time of manufacture (3). If little growth has occurred but, due to certain temperature relationships and other factors, there is a tendency for moisture droplets to unite during reworking, the bacterial cells in the larger moisture droplets should have more food available. With considerable bacterial growth in the butter and no tendency for collection of the moisture droplets during reworking, organisms should be distributed to more moisture droplets so that bacterial activity could be increased. With considerable growth and a tendency for aggregation of the moisture into larger droplets during reworking, the wide distribution of organisms to relatively large droplets should facilitate extensive bacterial development.

Because of the several possibilities, a variation in the effect of reworking butter is to be expected. While under certain conditions butter containing organisms capable of causing deterioration undoubtedly can be reworked without any harmful effect on the keeping qualities, under other conditions, particularly when there has been some multiplication of the organisms and the reworking aggregates the moisture droplets, reworking may definitely favor spoilage.

PROCEDURE AND METHODS

Because many of the trials involved the use of organisms producing serious defects in butter, most of the churnings were made with a hand churn, rather than with the churns in the butter laboratory, to avoid contamination of the large equipment and to limit the amount of defective butter. Occasionally, in studying changes in pH, butter made with butter culture was obtained, just before salting, from churns in the butter laboratory and worked by hand. All the butter was unsalted to permit more rapid growth of the organisms and was held at approximately 10°C. during the observations. Various trials indicated that with temperatures appreciably higher changes occurred too rapidly to be followed satisfactorily, and with temperatures appreciably lower some of the organisms produced changes very slowly.

The organisms employed were *Achromobacter lipolyticum*, *Achromobacter putrefaciens*, *Alcaligenes lipolyticus*, *Mycotorula*

lipolytica, *Pseudomonas fragi*, an unidentified proteolytic gram-negative rod capable of producing a putrid condition in butter (culture A) and various butter cultures. Except in the case of butter made in the butter laboratory from cream containing 5 to 7 percent butter culture, approximately 0.05 percent of a young milk culture was used to inoculate the pasteurized cream.

MANUFACTURE OF BUTTER

The butter was made from pasteurized cream according to the method employed by Long and Hammer (3). Usually under-worked and moderately worked butter were studied, but in some trials moderately worked and thoroughly worked butter were used.

REWORKING THE BUTTER

At various intervals, ranging from 1 to 7 days, portions of the butter were reworked with small, sterile, wooden paddles in the containers in which they were stored. One portion of each lot of butter was not reworked but was held as a control. During the reworking the butter was thoroughly mixed but was not reworked for excessive periods; in no case was there a conspicuous aggregation of moisture droplets.

BACTERIAL COUNTS

Bacterial counts were made by the plate method, using beef infusion agar and an incubation of 4 days at 21°C.

ACIDITIES

The pH of the butter serum was determined with the quinhydrone electrode, the serum being recovered by centrifuging melted butter and drawing off the fat.

The acidity of the fat was determined on a 10-gram portion with the procedure described by Breazeale and Bird (1).

EXPERIMENTAL

EFFECT OF REWORKING ON CHANGES IN NUMBERS OF BACTERIA IN BUTTER

In studying the effect of reworking butter on changes in numbers of bacteria, different organisms were used and the butter was reworked after various periods. Counts were made initially, at the time of reworking and at various intervals thereafter. In some of the trials the reworked butter showed approximately the same growth, or even less growth, than the control butter. These variations probably occurred partly because of errors inherent in the plate method of counting bacteria,

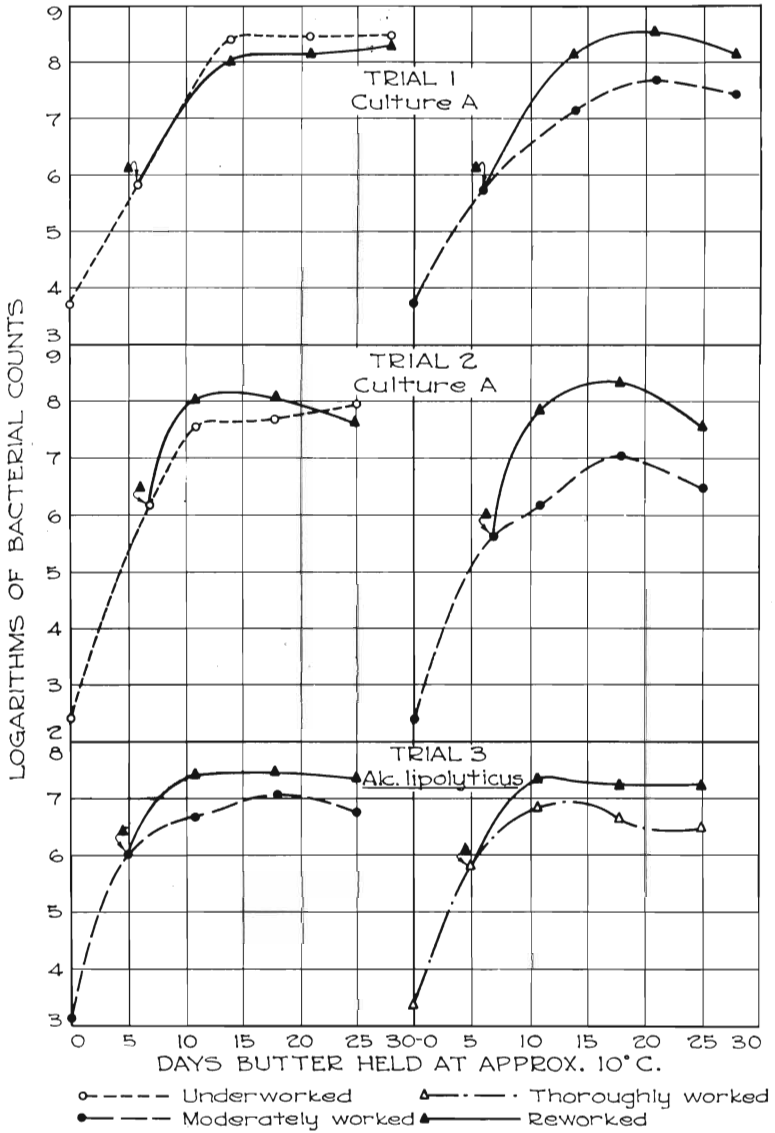


Fig. 1. Effect of reworking on changes in numbers of bacteria in butter.

among them being the failure of certain species to develop well on agar (4), and partly because there had been little growth of the organisms when the butter was reworked. As noted previously (3), plate counts on butter generally are not as satisfactory for showing the activity of bacteria as determinations of some product formed by them. In other trials reworking resulted in definite increases in the numbers of organisms. Three representative trials are presented in fig. 1, in which logarithms of bacterial counts are plotted against time. Trials 1 and 2 involved underworked and moderately worked butter, while trial 3 involved moderately worked and thoroughly worked butter.

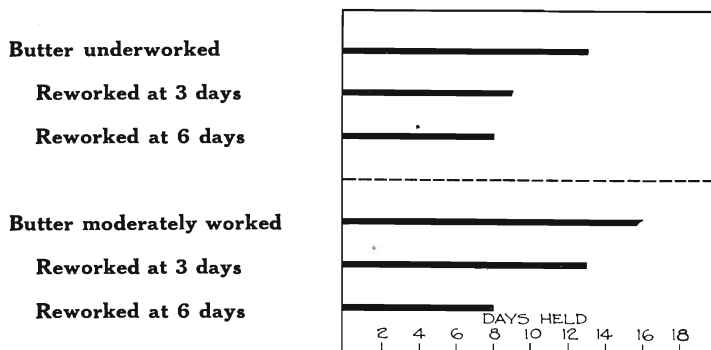
In the control butter the numbers of organisms after various holding periods were influenced by the extent of working (3), the numbers being lower with an increase in working. Reworking the butter after several days commonly caused a more rapid and more extensive increase in the numbers of bacteria. With the two samples of underworked butter, one showed an increase and one a decrease; with the three samples of moderately worked butter, all showed an increase; and with the one sample of thoroughly worked butter, there was an increase. At the last examination of each portion of butter, the count was essentially the same as it had been earlier, or was significantly lower, so it appears that multiplication of the bacteria in the butter was complete.

EFFECT OF REWORKING ON DEVELOPMENT OF DEFECTS IN BUTTER

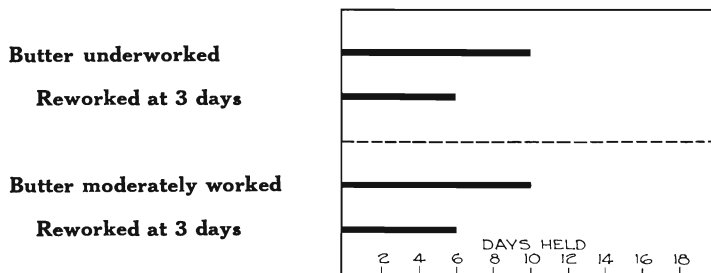
The effect of reworking butter on the development of defects was investigated with various organisms. The butter was reworked after different periods and examined frequently for the defect expected on the basis of the organism inoculated into the cream. Most of the trials involved underworked and moderately worked butter, although in a few instances moderately worked and thoroughly worked butter were studied. Results of representative trials are given in figs. 2 and 3.

With the control butter the usual effect of increased working was to increase the time required for appearance of a defect (3). The reworked butter generally spoiled in less time than the control butter. Although in some trials the control butter kept only a few days longer than the reworked butter, the differences are significant.

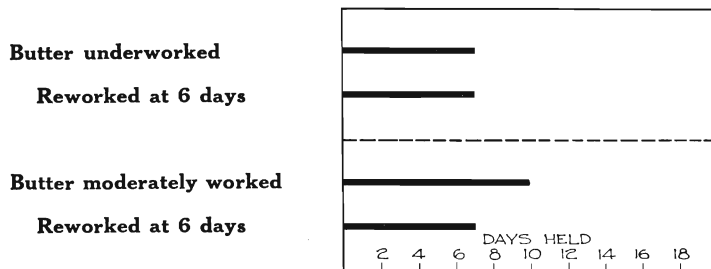
In each of the two trials involving reworking different portions of a lot of butter at different times, the butter which was reworked after the longer interval spoiled the faster. In one instance (underworked butter in trial 4) the portion which was reworked first kept longer than the control sample. Under



TRIAL 1. Days required for production of a putrid condition by culture A.

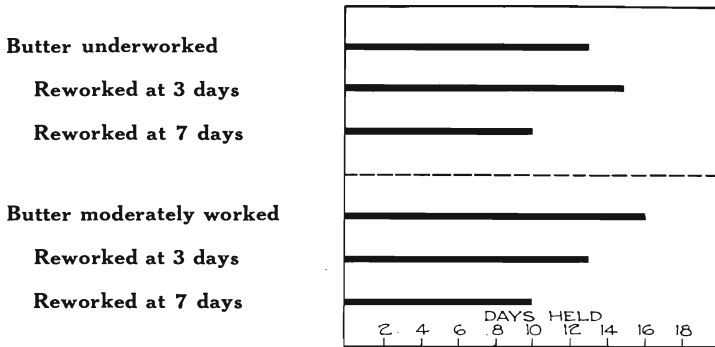


TRIAL 2. Days required for production of a putrid condition by *Ach. putrefaciens*.

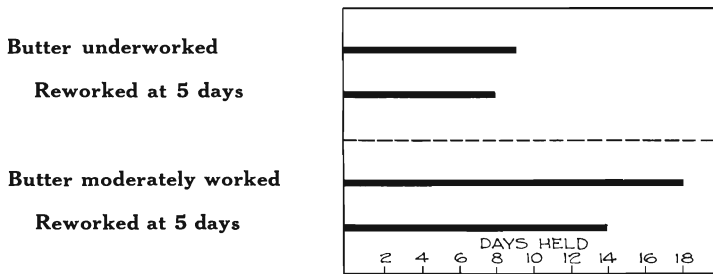


TRIAL 3. Days required for production of a rancid condition by *Ach. lipolyticum*.

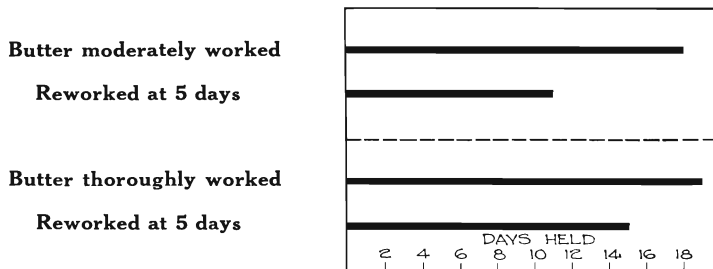
Fig. 2. Effect of reworking on development of defects in butter. The heavy lines show the periods the butter was normal.



TRIAL 4. Days required for production of a rancid condition by *Ps. fragi*.



TRIAL 5. Days required for production of a rancid to cheesy condition by *Myc. lipolytica*.



TRIAL 6. Days required for production of a rancid condition by *Alc. lipolyticus*.

Fig. 3. Effect of reworking on development of defects in butter. The heavy lines show the periods the butter was normal.

certain conditions this would be expected, because reworking before any considerable growth had taken place would fail to infect appreciably more moisture droplets and might tend to improve keeping qualities because of further dispersion of the moisture, especially with butter underworked originally.

In the instances in which reworking did not shorten the time required for spoilage, the defect was often more pronounced in the reworked butter than in the control, which is additional evidence of the greater activity of organisms following reworking.

EFFECT OF REWORKING ON CHANGES IN pH OF BUTTER SERUM

In investigating the effect of reworking on changes in pH of butter serum, butter cultures were used to inoculate the cream, and the butter was reworked after various periods. Determinations of the pH were made at once, at reworking and at different periods following the reworking. Some trials involved underworked and moderately worked butter, while others involved moderately worked and thoroughly worked butter. The results of five typical trials are presented in fig. 4, in which pH values are plotted against time. Trial 5 involved butter churned in the butter laboratory and worked by hand.

The more the control butter was worked, the slower were the changes in pH of the serum (3). Reworking regularly resulted in a more rapid change in pH of the serum, and this was evident at each examination. With trials 1 and 2 on underworked and moderately worked butter the differences were considerable, while in trial 3, also on underworked and moderately worked butter, the differences were less extensive. In trial 4 the control portion of the thoroughly worked butter showed relatively little change in pH of the serum throughout the holding period, while the reworked portion showed considerable change. The final pH value of the serum of the reworked portion was relatively high compared to underworked and moderately worked butter similarly treated, suggesting that thorough working of the original butter may tend to counteract the effect of reworking. Butter churned under commercial conditions with a relatively large amount of culture in the cream (trial 5) showed the same general changes in pH of the serum as butter made under laboratory conditions.

EFFECT OF REWORKING ON CHANGES IN ACIDITY OF FAT OF BUTTER

The effect of reworking on changes in acidity of the fat was studied with different lipolytic organisms, the butter being reworked after various periods. Acidities of the fat were deter-

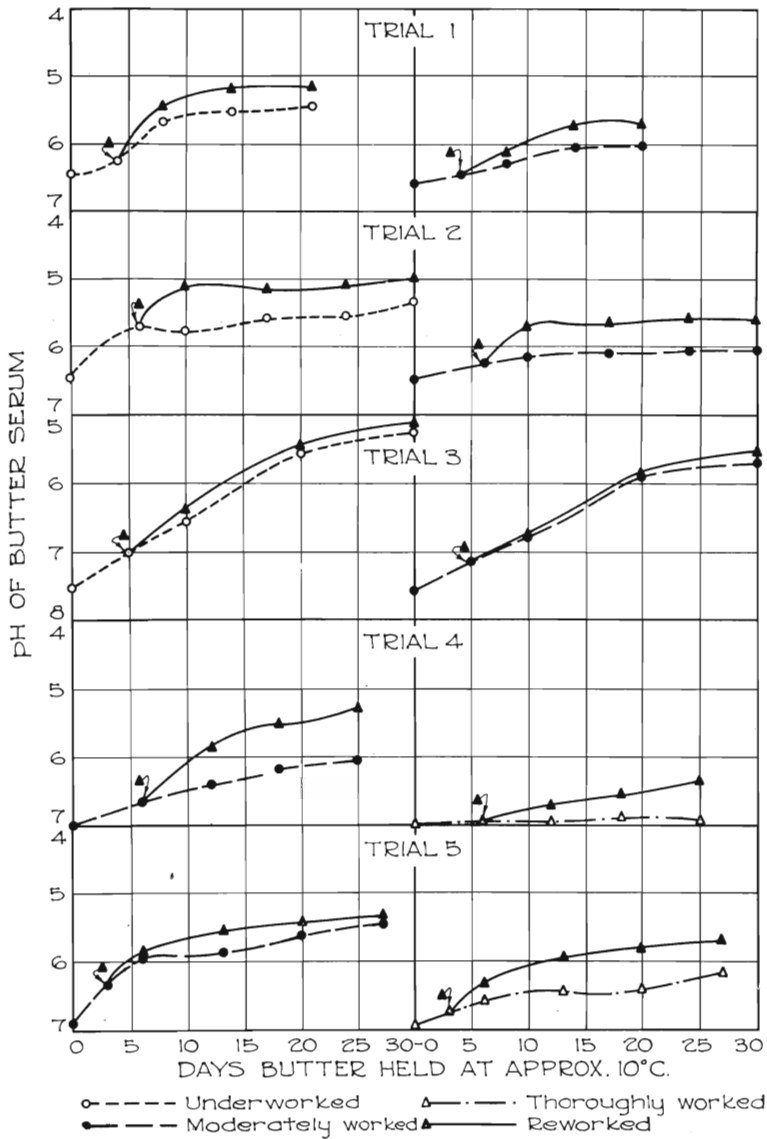


Fig. 4. Effect of reworking on changes in pH of butter serum.

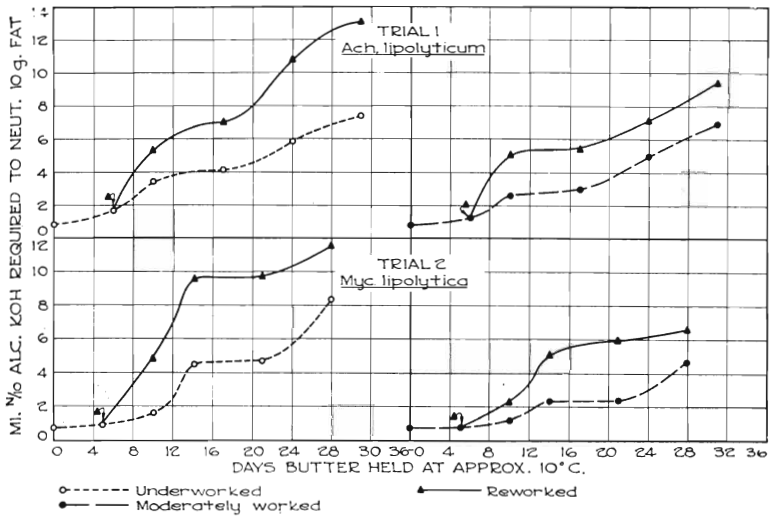


Fig. 5. Effect of reworking on changes in acidity of fat of butter.

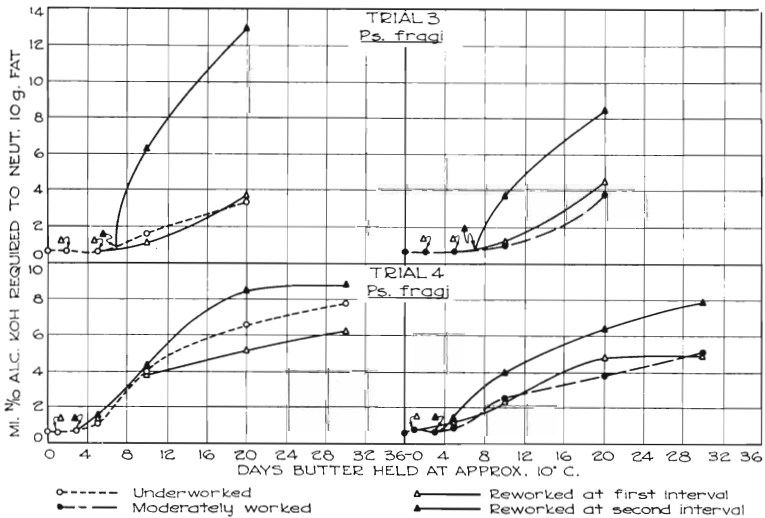


Fig. 6. Effect of reworking on changes in acidity of fat of butter.

mined initially, at reworking and at periods thereafter. The results of four representative trials on underworked and moderately worked butter are presented in figs. 5 and 6.

With the control butter the changes in fat acidity were nearly always less extensive in the moderately worked butter than in the underworked butter (3). Reworking of either type of butter after some growth of organisms had taken place resulted in more rapid increases in the acidities of the fat. In trial 1 with *Ach. lipolyticum* and in trial 2 with the yeast *Myc. lipolytica*, the reworked portions had considerably higher fat acidities than the controls at each examination following the reworking. In trials 3 and 4, involving reworking different portions of the same lot of butter at different times, the portion reworked after the longer interval always showed the higher fat acidity. With trial 3 the underworked and moderately worked portions which were reworked after 2 days had only slightly higher fat acidities at the last examination than the controls, while the portions reworked after 7 days had much higher values. In trial 4 the underworked portion reworked after 1 day showed a lower fat acidity than the control, while the moderately worked portion reworked after 1 day showed about the same value as the control. However, both the underworked and moderately worked portions reworked after 3 days showed much higher acidities than the control portions. The limited bacterial activity in the portions reworked early in the holding period is in agreement with the observations on development of defects.

GENERAL OBSERVATIONS ON REWORKING BUTTER

When butter accidentally contains more moisture than is legal, the elimination of a portion of it through reworking under certain temperature conditions is an established practice. Frequently, by forcing a portion of the moisture from butter and then draining it from the churn, the moisture content can be significantly reduced.

During the printing of butter with certain types of equipment, there is often a striking loss of water. Apparently moisture droplets are aggregated and forced from the butter in the same way that moisture is worked out of butter in a churn, the effect being greatest under certain temperature conditions. With butter poorly worked at the time of manufacture the losses are relatively large, while with butter well worked originally they may be negligible. The moisture that drains away is not the only moisture which is aggregated, and relatively large droplets are formed in the mass of butter. Even with well worked butter which shows no appreciable loss of water during printing, it cannot be assumed that the moisture dispersion is unaltered.

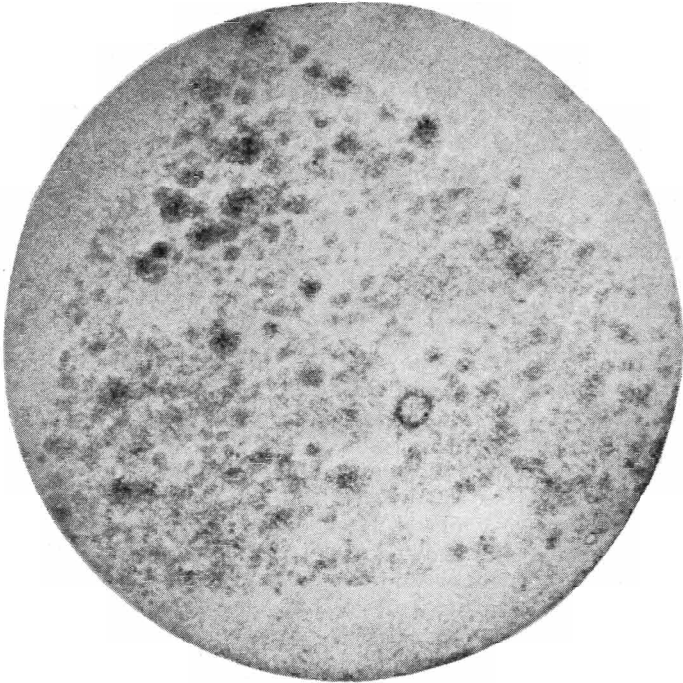


Fig. 7. Moisture distribution in a churning of rather poorly worked butter before printing (as shown by indicator paper).

The influence on the moisture distribution in butter of a printer having a reworking effect was studied with the indicator paper suggested by Knudsen and Sørensen (2). When the butter was taken from the tubs preparatory to printing, a fresh surface was exposed by cutting with a very fine wire and a piece of the paper was applied. After the butter had been through the printer a pound print was split with the wire and another paper applied to an exposed surface. The tests regularly showed that printing greatly influenced the moisture distribution. The influence was greater with poorly worked butter than with well worked butter but was very striking in either case. The general effect was evident without the use of the indicator paper, and the large moisture droplets developed rapidly on the freshly cut surfaces of the prints in a way which indicated that they were under considerable pressure. Figures 7 and 8 illustrate the effect of the printer on the moisture dispersion in a churning of rather poorly worked butter, as shown by the indicator paper.

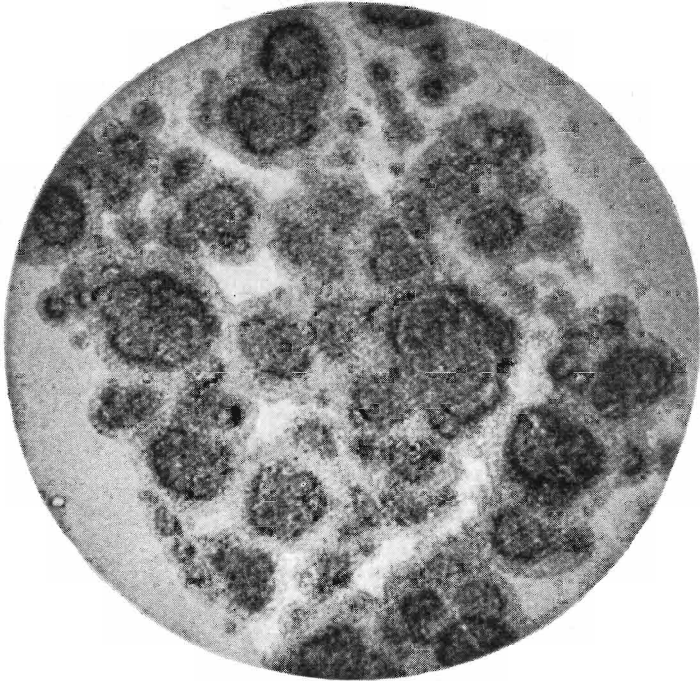


Fig. 8. Moisture distribution in a churning of rather poorly worked butter after printing (as shown by indicator paper).

DISCUSSION

The data indicate that under certain conditions the reworking of butter favors the growth of bacteria in it and accordingly accelerates various types of changes due to bacterial action. The effect of reworking undoubtedly depends on different factors, among which the extent of growth of bacteria in the butter before reworking and the general influence of the reworking on the aggregation of moisture are of importance.

The tests with *Myc. lipolytica* suggest that reworking influences the growth of yeasts in butter in essentially the same way as the growth of bacteria.

The effect of the reworking butter receives in certain types of printing equipment may explain the spoilage that has been noted occasionally in print butter when tub butter from the same churning has kept satisfactorily. Such an explanation is especially applicable in those cases in which the printer was in a good sanitary condition.

Although butter should be manufactured so that it does not contain organisms of importance from the standpoint of butter deterioration, the opportunities for contamination of butter with organisms of this general type are such that irregularities occur frequently. Under these conditions the possible effect of reworking butter, either in a churn or during printing, should be recognized.

The studies were carried out on unsalted butter in order to obtain extensive microbiological changes without using such long holding periods that factors other than the one being investigated would become important. The same effect of reworking would be expected with salted butter, although with this type of product the reworking would influence the distribution of salt as well as of moisture and thus complicate the interpretation of results. In the cases in which commercial butter has kept in tubs but not in prints, salted butter has been primarily involved.

LITERATURE CITED

- (1) Breazeale, D. F. and Bird, E. W. A study of methods for the determination of acidity in butter fat. *Jour. Dy. Sci.*, **21**:335-344. 1938.
- (2) Knudsen, S. and Sørensen, A. Über eine Methode zur Beurteilung der Grösse der Wassertröpfchen in Butter. *Fette u. Seifen*, **45**:669-670. 1938.
- (3) Long, H. F. and Hammer, B. W. Bacteriology of butter. VI. Effect of moisture dispersion in butter on growth of bacteria. *Iowa Agr. Exp. Sta., Res. Bul.* 246. 1938.
- (4) Long, H. F. and Hammer, B. W. Examination of butter with the Burri smear culture technic. *Iowa State Col. Jour. Sci.*, **12**:441-450. 1938.