Bacteriology of Butter

III. A Method for Studying the Contamination From Churns

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SUMMARY

A method for the study of the contamination from churms is suggested. It consists of allowing a small amount of an agar medium containing 2.5 percent air-dried agar to solidify in contact with the surface to be studied, the transferring of the agar preparation thus formed to a sterile petri dish and finally the counting of the colonies that develop on incubation. With surfaces nearly horizontal the agar is poured on, while with surfaces not nearly horizontal the medium is poured behind a glass plate held a short distance from the surface by a gasket. The results are expressed as the number of colonies developing per square centimeter of the agar preparations.
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BY B. W. HAMMER AND H. C. OLSON*

The equipment with which dairy products come in contact has long been recognized as an important source of the microorganisms present in these materials. With butter, churns are especially important in this connection because the surface involved is nearly all of wood instead of metal, which complicates both the washing and the treatment intended to destroy the microorganisms remaining after the washing.

The studies carried out at the Iowa Agricultural Experiment Station on the bacteriology of butter have emphasized the importance of churns as a source of bacteria which are capable of bringing about objectionable changes in this product. The contamination of butter from all sources is undoubtedly of greater significance than formerly, due to the present tendency to extend the manufacture of butter without salt or with a low salt content. The salt in butter has a very definite effect in restraining bacterial growth and, when the percentage is comparatively high, may be responsible for a decrease in bacterial content that extends over a considerable period even with holding temperatures that are very favorable for the growth of bacteria.

A procedure that has been widely used in studying contamination from dairy plant equipment is to expose water or milk to the equipment and then determine the bacterial content; if the material used is not sterile it is, of course, necessary to determine the bacterial content before the exposure so that the organisms added can be calculated. This general plan is very useful for many purposes. For example, in the case of market milk a series of samples taken at various points during the regular processing may give a detailed picture of the plant contamination that leads up to the count on the finished product.

The contamination from a churn during its actual operation is difficult to determine because, (a) cream is added to the churn while butter is removed, and the separation of the fat influences the distribution of the organisms so that there are many more organisms per milliliter of buttermilk than per milliliter of butter; (b) the material is in contact with the churn so long that the multiplication of organisms may occur; (c)

*H. C. Olson holds a fellowship provided at the Iowa State College by the Iowa State Brand Creameries, Inc., of Mason City, Iowa.
the agitation in the churn is so vigorous and extended that the breaking up of clumps would be expected to have an unusual influence on counts made by the plate method, and this is the only procedure that can be employed logically because, as a result of the pasteurization of the cream, a large percentage of the microorganisms is dead.

The determination of the number of bacteria picked up by water used to rinse out a churn undoubtedly gives useful information on the condition of the churn. It takes considerable quantities of water, however, to completely cover the inside of a churn and the sterilization of these quantities is rather inconvenient. Without sterilization counts must be made on the water both before and after it is used in the churn; this would not be as objectionable if the water were to go thru other equipment so that the count on the water coming from the churn would provide the initial count for another determination, but without this arrangement two counts are necessary for a result on one piece of equipment.

A PROCEDURE FOR STUDYING THE CONTAMINATION FROM CHURNs

In connection with studies on churn contamination, a procedure has been developed which appears to be useful in securing a general idea of the condition of a churn from the standpoint of the presence of microorganisms. It involves allowing a small amount of a special agar medium to solidify in contact with the surface to be studied, the transferring of the disc thus formed, to a sterile petri dish, and finally the counting of the colonies that develop on incubation. The general steps in the method are as follows:

1. A tube of a special agar medium (about 10 ml.) is melted and cooled to from 41 to 43 degrees C.
2. A small area on the surface of the interior of the churn is moistened with sterile water, using a sterile cotton swab.
3. The medium is poured on the moistened area and allowed to solidify.
4. The disc thus formed is picked up with a sterile spatula and tipped into a sterile petri dish so that the side of the disc which was next to the wood will be toward the top of the dish.
5. The disc is incubated at 21 degrees C. for four days; in case the colonies are so numerous that there is danger of them growing together the incubation period is reduced.
6. The colonies appearing on a measured area of the disc are counted and the results expressed as the number per square centimeter.
DEVELOPMENT OF THE MEDIUM USED

An agar made with beef infusion was selected for the study of the general contamination from churns because a medium of this type has been used rather regularly at the Iowa Agricultural Experiment Station in investigations on the organisms important in the deterioration of butter. Media of other types may, of course, be used and under certain conditions may be preferable.

Beef infusion agar containing 1.5 percent of air-dried agar was not satisfactory because a disc of a suitable size (5 to 8 cm. in diameter) prepared with it usually broke when an attempt was made to remove the disc from the surface with a spatula. Beef infusion media containing higher concentrations of air-dried agar—2.0, 2.5, 3.0 and 3.5 percent—were then tried with the idea of finding a medium which would yield discs capable of surviving the handling necessary. A number of trials indicated that a medium containing 2.5 percent air-dried agar would give discs which could be picked up from a surface and transferred to a sterile petri dish with a minimum loss. Media with higher agar concentrations also gave satisfactory discs, but the addition of agar beyond 2.5 percent seemed unnecessary, and a concentration of 2.5 percent was accordingly adopted."

Attempts were made to reinforce the discs prepared from the medium containing 1.5 percent agar with cheesecloth.

*Beef infusion agar containing 2.5 percent air-dried agar is somewhat more difficult to prepare than that having the usual percentage of agar. A procedure that has been found convenient is to make up the medium with 1.5 percent air-dried agar and then add the remaining 1 percent agar and incorporate it by autoclaving, altho with this method the additional agar is not subjected to the clearing effect of the meat constituents that coagulate with heat.

Fig. 1. Equipment used with the method.
chiffon and similar materials. The usual procedure was to place a sterilized disc made from one of these materials over the area to be covered and then pour on the melted agar. Fairly satisfactory preparations were secured in this way, but often large air bubbles were held in the agar, and there was also difficulty in counting the colonies against the more or less ununiform background. Discs of such materials as cellophane, thru which the agar could not pass, commonly contained large air bubbles. In general, the use of a reinforcing disc seemed a much less satisfactory procedure than the use of a medium containing 2.5 percent air-dried agar.

DETAILED STEPS IN THE METHOD FOR SURFACES NEARLY HORIZONTAL

1. The medium to be used is stored in test tubes, approximately 10 ml. to each. The tubes of medium needed for the churn or churns to be examined are melted, cooled in water to 41 to 43 degrees C. and then held at this temperature.

2. The preparation of a disc can be carried out most satisfactorily by having the surface of the churn dry and then moistening, with sterile water, the exact area to be covered just before the agar is poured on. When the entire surface is wet the medium spreads very rapidly, and the shape and size of the area over which the agar flows cannot be controlled. As a result the disc may be of an unsatisfactory shape, and it is likely to be so thin that it cannot be handled without breaking. On a surface that is thoroly dry, the agar adheres so

Fig. 2. Pouring the agar on a moistened area
firmly that the disc cannot be removed easily and tearing is common; presumably the agar does not break away from the surface readily because it has penetrated irregularities in the surface of the wood. A circular area 5 to 8 cm. in diameter is moistened with sterile water by means of a sterilized cotton swab (see fig. 1) which has been dipped into the water. The swab may be prepared by wrapping a small amount of absorbent cotton around a piece of wood or stiff wire, putting this into a test tube, stoppering the test tube and then sterilizing. The water is also conveniently sterilized in a test tube.

3. The mouth of the test tube containing the agar is thoroly flamed and the agar then poured on the moistened surface rather slowly (see fig. 2). If the surface is nearly horizontal the medium will readily spread over the moistened area. In case the surface is not approximately horizontal, as shown by the spreading of the agar, it may be levelled by tilting the churn slightly and the thickness of the disc thus kept reasonably uniform. The agar will solidify in a very short time if the churn is cool and the churn can then be tilted so that the disc can be removed conveniently.

4. The disc (see fig. 3) is picked up by means of a sterile spatula. The first step is to loosen the edge of the disc by placing the spatula almost flat against the surface and running it under the edge of the disc. Unless this is done a portion of the edge may break off when the disc is lifted. The spatula is then slowly forced under the disc, care being taken to keep it as flat against the surface as possible (see fig. 4). When the disc is free it is tipped into a sterile petri dish so that the portion of the disc which was exposed to the wood will be toward the top of the dish.
A spatula with a thin, flexible blade that is rather large and rounded at the tip (see fig. 1) has been found most satisfactory. There should be no sharp edges since these tend to cut into the disc. The spatula may be wrapped in paper or put into a metal container and sterilized; a metal container with an easily removable lid, such as a pipette case, is especially satisfactory when a number of spatulas are to be sterilized. A thin coating of vaseline or similar material will largely prevent the rusting of the blade during the sterilization and subsequent holding of the spatula.

5. The usual incubation for the disc is 21 degrees C. for four days. With a badly contaminated churn the colonies will be so numerous that they may grow together in this period, and it is accordingly advisable to examine the disc from day to day and consider the incubation complete in less than four days if the colonies are beginning to fuse.

6. At the end of the incubation period the colonies on a measured area are counted. A convenient area, which depends on the size and shape of the disc, is ruled off in square centimeters on the bottom of the petri dish with a wax pencil (see fig. 5). The colonies within this area are counted, using a hand lens; with a large number of colonies many are so small, due presumably to the crowding, that they are likely to be missed unless a lens is used. The number of colonies per square centimeter is calculated from the number counted and the area over which these are distributed.

Fig. 4. Removing an agar disc from a churn shelf.
When the number of colonies per square centimeter is very large, it is necessary to depend on approximate counts or even estimates because of the difficulty involved in counting the closely packed colonies. With a large number of colonies on a preparation, there may be a distinct advantage in ruling the desired area into portions smaller than 1 sq. cm., for example into areas of $\frac{1}{4}$ sq. cm.

**THE METHOD FOR SURFACES NOT NEARLY HORIZONTAL**

While undoubtedly a fairly satisfactory idea of the microbiologic condition of a churn can be secured by preparing discs on surfaces that can be got nearly horizontal (e. g. a shelf, a roller, a door, etc.), it seemed desirable to develop a procedure that could be used on surfaces, such as the ends of the churn, that are not nearly horizontal. The method employed involves the pouring of the special medium behind a glass plate held a short distance from the surface by a gasket so that an agar preparation, comparable to the disc obtained on a nearly horizontal surface, can be secured.
Since it appeared that an agar preparation with an area of 20 sq. cm. would be satisfactory, the glass plates and gaskets were designed on this basis. Glass plates approximately 5 x 6.5 cm. were cut from heavy window glass. The glass must be heavy so that it can be held firmly against the surface without danger of breaking or of part of it being forced close to the surface to be studied. The gaskets were made from thick (2 to 3 mm.) pulp board, secured from strong packing boxes, by cutting out rectangles 6.5 x 8.5 cm., removing a rectangular section 4 x 5 cm. from each so that a border 1.25 to 1.75 cm. in width was left and then cutting a section out of the border at one end (see fig. 1) to provide an opening thru which the medium could be poured. The gaskets must be made from pulp board which will retain its shape on sterilization; some of the board tried split into layers when it was heated. The cut surfaces on the gaskets with which the agar comes in contact should be very smooth so that the agar can be split from them easily. The glass plates and gaskets may be sterilized after wrapping in paper, or the glass plates may be sterilized in a petri dish and the gaskets in a beaker covered with half of a petri dish.

The exact procedure used for securing an agar preparation from a vertical surface is as follows: A circular area about 7 cm. in diameter is moistened with sterile water using a sterile swab. The sterile gasket is removed from its container by means of sterile forceps and placed over the moistened area so that the end of the gasket from which a section has been

Fig. 6. The end of a churn with a gasket and a glass plate held ready for the agar.
removed is at the top. The glass plate is taken from its container with sterile forceps and placed over the gasket. With the glass plate held firmly against the gasket (see fig. 6), the agar is poured thru the opening in the gasket until the enclosed space is full. The whole is held firmly in place until the medium is thoroly solidified. If the glass plate is chilled in a refrigerator just previous to use and the churn is quite cool solidification requires only a short time. With a clean churn the firm agar will ordinarily hold the glass and gasket in place (see fig. 7), but when the churn has been carelessly cleaned so that the surface has retained some fat the preparation may fall unless it is supported. The glass plate is removed by slipping it along the gasket, after which the gasket is removed by running a small sterile spatula between the agar and the gasket and lifting the gasket from the surface of the churn. The agar is then removed with a sterile spatula and put into a sterile petri dish, so that the portion which was next to the wood is toward the top of the dish.

The agar preparations are incubated and the colonies that develop are counted by the methods already given for the discs prepared on nearly horizontal surfaces.

OBSERVATIONS ON THE METHOD

The best results are obtained with the method outlined when the surface of the churn is cool and dry. Unless the churn
has cooled thoroughly following washing or rinsing with hot water, the medium, on a surface nearly horizontal, is likely to spread over a considerable area before it solidifies and to yield a disc which is too large and too thin to transfer to a petri dish without breakage. Frequently such a disc can be used by trimming it down with a sterile spatula or scalpel and transferring the desired portion. A reasonably cool churn is also important when a preparation is to be secured from a surface that is not horizontal because the glass plate and gasket must be held in place until the medium is completely solidified. Moisture over the whole surface to be studied instead of only over the portion to be used for the preparation also tends to make the medium spread too much in the case of a surface nearly horizontal and, accordingly, causes the same general difficulties as a warm surface.

In the comparisons carried out, the numbers and types of microorganisms from various portions of a churn have been much the same, but some variation has been noted, and it seems advisable to secure preparations from several surfaces unless only general results are desired. In the variations encountered there was a tendency for the numbers of organisms per square centimeter to be slightly less on the ends and rollers than on the shelves and barrels. This relationship may be determined by such factors as the following: (1) a roller is ordinarily of a different type of wood than the barrel and shelf; (2) when the door of a churn is open the ends are probably subjected to less air contamination than a shelf, a roller, etc. It appears from the results secured that, in general, the churns or portions of a churn having a smooth surface contain fewer organisms than those with a rough surface.

It would be expected that the pouring of the agar on a moistened surface would dislodge a considerable portion of the organisms and distribute them throughout the medium. With the preparations that have been secured, however, nearly all of the colonies have developed on the agar surface that was exposed to the wood and comparatively few colonies embedded in the medium have been encountered.

A number of trials were carried out in which several discs—one after another—were secured as rapidly as possible from the same area. These showed very definitely that by no means all of the organisms are picked up by the medium poured on a wood surface. In general, there was a fairly regular decrease in the number of organisms present throughout the series of discs from an area, but some organisms were still picked up after several discs had been prepared.

In a number of trials the results obtained with the method suggested were compared with the counts secured on sterile
water used to rinse out the churn. Agar preparations were first made from various places in a churn with the procedure described and then 10 gallons of sterile water were revolved in the churn for 15 minutes after which this rinse water was plated, using beef infusion agar (1.5 percent air-dried agar) and an incubation of four days at room temperature. The data obtained are given in Table I. These show that there is a general relationship between the results secured with the two procedures but that the relationship is by no means a close one. With churn A the ratio between the number of colonies per square centimeter of the agar preparations and the number per milliliter of rinse water varies from 1 to 1,051 to 1 to 4,167 and for churn B it ranges from 1 to 222 to 1 to 8,097. The churn with which the highest ratio was secured had stood for one day after being poorly drained so that there undoubtedly was a good opportunity for the growth of organisms. The ratio between the average number of colonies per square centimeter of the agar preparations (for the 15 trials) and the average number of organisms per milliliter of rinse water (for the 15 trials) is 1 to 1,650.

### Table I. Comparison of the Results Secured (1) by the Method Suggested and (2) by Counting the Number of Organisms in Water Used to Rinse Out the Churn

<table>
<thead>
<tr>
<th>Churn</th>
<th>Date</th>
<th>Agar discs</th>
<th>No. of colonies per ml. of rinse water</th>
<th>Ratio of colonies per sq. cm. of agar discs to colonies per ml. of rinse water</th>
<th>Remarks on condition of churn</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Jan. 24</td>
<td>3</td>
<td>2.7</td>
<td>11,250</td>
<td>1 to 4,167</td>
</tr>
<tr>
<td>A</td>
<td>Jan. 28</td>
<td>3</td>
<td>23.9</td>
<td>38,000</td>
<td>1 to 1,589</td>
</tr>
<tr>
<td>A</td>
<td>Jan. 29</td>
<td>3</td>
<td>18.9</td>
<td>38,000</td>
<td>1 to 3,068</td>
</tr>
<tr>
<td>A</td>
<td>Feb. 5</td>
<td>2</td>
<td>27.3</td>
<td>28,700</td>
<td>1 to 1,051</td>
</tr>
<tr>
<td>A</td>
<td>Feb. 26</td>
<td>4</td>
<td>14.2</td>
<td>37,500</td>
<td>1 to 2,641</td>
</tr>
<tr>
<td>A</td>
<td>Jan. 14</td>
<td>5</td>
<td>8.6</td>
<td>33,000</td>
<td>1 to 6,244</td>
</tr>
<tr>
<td>B</td>
<td>Jan. 25</td>
<td>6</td>
<td>12.7</td>
<td>41,000</td>
<td>1 to 2,228</td>
</tr>
<tr>
<td>B</td>
<td>Jan. 28</td>
<td>5</td>
<td>9.8</td>
<td>35,000</td>
<td>1 to 3,571</td>
</tr>
<tr>
<td>B</td>
<td>Mar. 3</td>
<td>6</td>
<td>27.0</td>
<td>1,000</td>
<td>1 to 222</td>
</tr>
<tr>
<td>B</td>
<td>Mar. 5</td>
<td>6</td>
<td>12.7</td>
<td>41,000</td>
<td>1 to 2,228</td>
</tr>
</tbody>
</table>

| Average of 15 trials | 54.2 | 89,405 | 1 to 1,650 |

A close relationship between the results of the two procedures would not be expected if what is being measured by each is considered. In the case of the agar preparations there is no reason to believe that each colony develops from a single bacterial cell and, as has already been shown, by no means all of the organisms are picked up from the wood by the agar. The colonies developing from the rinse water probably include
many that represent a group of organisms, and the rinse water cannot be expected to dislodge all the organisms from the surface of the churn.

GENERAL RESULTS OBTAINED WITH THE METHOD

A considerable number of agar preparations have been secured from churns washed in various ways. In general, comparatively low bacterial counts per square centimeter have been obtained on churns given thorough treatment in the washing and in the attempt at sterilization; on the other hand, comparatively high counts have been secured on churns carelessly treated. These results have been obtained with the churns used in studying the effect of different treatments on the bacterial content and also with the churns in operation in various plants in the state. The colonies present in preparations made from churns given a thorough treatment usually suggested very few types of organisms and often included primarily bacteria belonging to the genus Bacillus, while those present on preparations made from churns carelessly handled commonly suggested a varied flora which often included micrococci producing yellow colonies on the medium. Yeasts and molds were distinctly more numerous in the preparations from carelessly treated churns than in those given thorough treatment. One of the churns used in studying various methods of handling could be got in such a condition by thorough washing and rinsing with hot water that the preparations secured from it suggested a pure culture of a species of the genus Bacillus.

In the examination of a large number of churns with which various treatments had been used, the counts ranged from less than 1 to more than 1,000 (estimated) per sq. cm.

ADVANTAGES OF THE METHOD

The method suggested gives a general picture, from the standpoint of the microorganisms present, of the churn surface with which the cream and butter come in contact. The results secured appear to be more understandable by butter plant employees and more applicable to the problem of contamination from churns than the results secured when a churn in rinsed with a quantity of water and a bacterial count made on the water.

Comparatively little equipment is necessary to carry out the procedure, all of which is readily available and easily transported. The preparations are completed at the churn itself, and it is not necessary to take rinse water or some such
material to a laboratory and work with it there. For these reasons the method is particularly adapted to work in the field where it may be desirable to examine churns in a number of plants on one trip. Moreover, the temperature of incubation suggested is such that during much of the year ordinary room temperature is quite satisfactory.

The agar preparations, when properly made, show the irregularities in the surface covered, such as the grain of the wood. Some of the preparations secured have had parallel rows of colonies that followed the grain of the wood and thus apparently showed the particular portions of the surface from which the largest number of organisms were coming. While this is of no great advantage from the standpoint of the cleaning of the churn, it does tend to emphasize the greater difficulties to be expected in the cleaning of a churn than in the cleaning of a metal surface.

THE USE OF THE METHOD FOR YEAST AND MOLD COUNTS ON CHURNs

The method suggested was originally intended for the study of the bacteria in churns, but many species of yeasts and molds develop on the medium used. While the mold colonies are rather easily differentiated, the differentiation of the yeast colonies would require detailed microscopic study of the colonies or preferably of stained preparations made from them. The method can be applied directly to the study of yeasts and molds in churns by using a medium comparable to those employed in the determination of yeasts and molds in butter and containing 2.5 percent air-dried agar; in this manner the development of bacterial colonies can be largely controlled. In the examinations that have been made the numbers of yeasts and molds in churns have always been much smaller than the numbers of bacteria. This relationship, together with the much greater importance of bacteria than of yeasts and molds as a cause of the usual deterioration in butter, suggests that the determination of the numbers of yeasts and molds in a churn is not a sufficiently rigid test and that if satisfactory churn treatment is to be established by microbiologic examinations, the bacteria, rather than the yeasts and molds, should be investigated. In the studies that have been carried out yeasts and molds have been most numerous on preparations made from churns exposed to air contamination for periods of several days by leaving the open door up.
APPLICATION OF THE METHOD TO EQUIPMENT OTHER THAN CHURNS

Altho the method suggested was developed primarily for the examination of churns, satisfactory preparations have also been secured from pails, cans, weigh vats, cooler troughs, ice cream freezers, etc., and it appears that the method can be adapted to almost any type of equipment. Small pieces, for example pails and cans, are easily put in such a position that the surface to be examined is nearly horizontal.

The solidified agar can be removed from a metal surface very easily and it is not necessary to moisten the area over which the agar is to be poured. With preparations secured from metal surfaces there appeared to be more of a tendency for spreaders to develop than with preparations secured from wood surfaces; this may have been due to the types of organisms present on the metal surfaces examined.

Fig. 8. Preparation from the end of a churn in a very unsatisfactory condition.
Fig. 9. Preparations from the shelf of a churn in an unsatisfactory condition. Many tiny colonies along the grain in the wood.

Fig. 10. Preparation from the shelf of a churn in an unsatisfactory condition. Many small colonies are present among a comparatively few large colonies.
Fig. 11. Preparation from the shelf of a churn in a fairly satisfactory condition.

Fig. 12. Preparation from the end of a churn in a fairly satisfactory condition.
Fig. 13. Preparation from the end of a churn in a rather unsatisfactory condition.

Fig. 14. Preparation (trimmed) from the shelf of a churn in a good condition.
Fig. 15. Preparation from the shelf of a churn in a very satisfactory condition.

Fig. 16. Preparation (trimmed) from the shelf of a churn in a very satisfactory condition.
Fig. 17. Preparation (trimmed) from the shelf of a churn in a very satisfactory condition.

Fig. 18. Preparation (from the roller of a churn) showing a number of mold colonies among the bacterial colonies.
Fig. 19. Preparation from the shelf of a churn in a well managed Iowa creamery.

Fig. 20. Preparation from the end of a churn in a well managed Iowa creamery.