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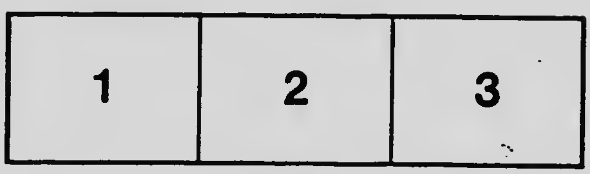
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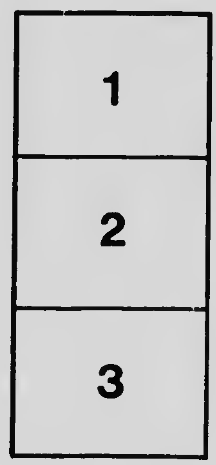
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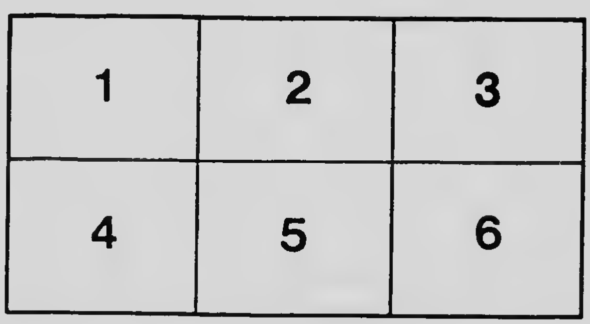
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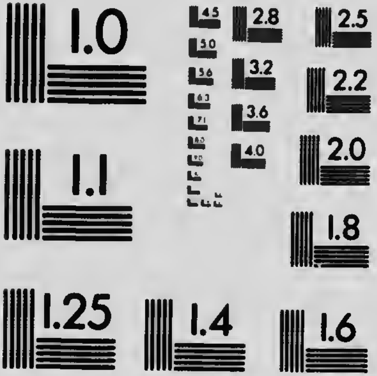


BULLETIN 120 MAY, 1902
Ontario Agricultural College and Experimental Farm.

BITTER MILK AND CHEESE.

By F. C. Harrison, Professor of Bacteriology.

During the past summer one of the leading cheese factories of the Province was very much troubled with an affection known as *bitter milk*, which gave a very unpleasant flavor to the cheese. The trouble was not a new one at this factory. The year before last it caused some annoyance and loss, but not so much as last year; and even two years ago it was occasionally noticed; hence it would seem that the infection was increasing from year to year.

The patrons of the factory could not account for the trouble. They had been handling their milk the same way for years and had no trouble until a few years ago. Hence some were disposed to blame the maker. Others that had no silos blamed those who had, thinking that ensilage gave a bitter taste to the milk; and others again regarded Ragweed (*Artemisia ambrosifolia*) as the cause of the unpleasant flavor. None thought that the true cause might be a micro-organism,—because neither patrons nor makers have yet begun to realize the close relation of bacteria to dairying in all its branches.

This and similar troubles are constantly on the increase. J. W. Robertson (1), the Canadian Dairy Commissioner, states that during the first year that the creameries in the North-West Territories were operated there were no bad flavors; that, in fact, it was difficult to get proper souring; but now, not only does the ordinary souring progress in the usual manner, but also a number of bad and "off" flavors are met with. In Eastern Ontario, we have records of Rusty spot in cheese; and in New York, a number of troubles, such as Sweet Flavor in Cheddar cheese and Fishy flavor in milk.

Judging from the varied complaints and the increasing number of samples sent to the Bacteriological Laboratory for examination, it is evident that a serious condition of affairs exists, and that the methods of handling milk, as practised on the average farm, must be changed, if we are to improve or even maintain the reputation of our dairy products. Nor should the improvement be confined to the patron; for many factories also need improvement, the commonest defects, according to J. A. Ruddick of the Dominion Department of Dairying, being bad floors and drainage, cheap apparatus, and improperly constructed curing-rooms. These rooms become too warm in summer and give rise to the so-called "heated flavors." The chief defect from the maker's standpoint, according to the same authority, is a weak, open body in the cheese, due to insufficient development of acidity during the process of manufacture.

I may also add that this lack of acidity not only causes open cheese, but sometimes gives rise to bad flavors as well, for the acid checks the development of many injurious bacteria that may be present in the cheese.

In August, 1901, we were asked by a large Western factory to conduct a series of tests in order to ascertain the cause of the bitterness in the milk. During that month the factory had bitter milk nearly every day, although it was very difficult to detect by smell at the time the milk was dumped into the weigh-can ; but, in exceptional cases, the bitterness could be detected even then. The bitterness could be more easily smelt when the vat was warmed up for setting ; and the odor increased as the curd was heated. When the curd was very bitter, the top of the whey foamed with small gas bubbles, especially when stirred.

The following notes on the making at the factory were supplied to me by Mr. Stratton, the College cheesemaker, who accompanied me on one of my visits:

CULTURE. About one-half of one per cent. culture was added to each vat at the time of commencing to fill with milk.

CONDITION OF MILK. Most of it seemed in fair condition, but on a few cans the so-called "bitter flavor" could be detected.

SETTING THE MILK AND HEATING THE CURD. The milk was ready to heat soon after heating up (average per cent. acid, per alkali test, .182). The agitators were set in motion soon after the cutting of the curd ; then, after the temperature was raised to 98°, a hand rake was used. During the process of heating, the flavor became very pronounced on all the curds.

DIPPING. Most of the curds were dipped early. The whey w[illegible] off when the acid showed about ⅜ in. on the hot iron (average per [illegible] test, .210). The stirring was continued from 15 to 20 times, acc[illegible] to the condition of the curd. The acid developed so fast on some that it would draw out about one inch. Before the stirring was completed, the curds showed also a correspondingly high acid per alkali test.

MILLING. The curds were ready to mill in a little over two hours from time of dipping (average alkali test, 1.152). After milling, they were washed with about eight pails of water at a temperature of 98°.

MATURING AND SALTING. When freshly milled, the flavor is strongest ; but there seems to be a permanency about the bitter flavor ; and there is little improvement while the curd is maturing. The mellowing down process is also very slow. Probably this is quite largely due to the excessive amount of stirring required to dry the curds at dipping time. In some vats the bitter flavor was more pronounced than in others, and was occasionally accompanied by an aromatic flavor, or fruity flavor, especially noticeable when one pulled up the cheese cloth to turn the curds at the end of the vat. The curds at salting time had a fine, mellow, velvety feeling, although the change during the last hour and a half or two hours was slight. The curds showed a very high acid all through. Two curds tested after milling and again just before salting, showed a gain of only .034 per cent. acid.

Samples of the bitter curd were brought to the laboratory, and among the micro-organisms separated was a yeast like species which produced a bitter taste in milk. Large numbers of other varieties of yeasts were present, some of which gave pleasant, ethereal odours when grown in beerwort.

FIG. 1.—Milk cans kept under trees. On the leaves of fruit and other trees many species of micro-organisms are found, and this illustrates how they get into the cans.

From the cultures obtained from the curd starters were made and added to the milk, and small experimental cheese were made therefrom. One of these cheese had a flavor which was very similar to, if not identical with, that of the cheese in the factory referred to, and, having thus succeeded in demonstrating that the bitterness could be produced at Guelph, and that it was due to a form of yeast, we decided to analyse bacteriologically samples of the mixed milk from every patron of the factory, to ascertain if the ' bitterness " came from the milk furnished by certain individuals, or if it was a general infection.

Samples of the milk of each person were taken in sterilized bottles as it was poured from his can into the weighing can. These were then packed in a box and sent to the laboratory. They arrived there in good condition. No bottles were broken and no corks were blown out. Cultures in beer-wort and beer-wort gelatine were made from each sample. The sample itself was placed in the incubator at 37.5° C. for 24 hours;

and at the end of that time it was examined for gas, digestion of curd, and flavor. The wort cultures were also incubated at 37° C. for 48 hours, and then examined microscopically for the presence of yeasts. The wort gelatine plates were kept at 20° C., and, as the colonies developed, they were studied and, if necessary, isolated. In this way 99 samples of milk, from as many different patrons, were examined, and yeasts were found in 97, in many cases several species being present; but the predominant variety was the one giving rise to bitter milk, to which I have given the name of *Torula amara*, or the bitter Torula. *B. coli*, *B. lactis aerogenes*, *B. subtilis* were also present in the majority of samples; and the following species of bacteria and moulds were also quite common: *Oïdium lactis*, *Penicillum glaucum*, *Proteus*, *Saccharomyces anomalus*, the pink Torula, and a number of species that were not determined, viz., yellow chromogenic forms, a red mould, a capsulated bacillus, various cocci, various yeasts, a slime producing bacillus, etc.,—a rich and diverse flora.

In fact, the bacterial flora was so rich that it was thought advisable to visit all the patrons, examine the conditons under which the milk was produced, and endeavor to locate the habitat of the bitter torula. The farms of some 82 patrons were visited. Samples of the foremilk (or first milk drawn from each teat) of a number of cows were taken; also of the can and pail washings; and, if the milking was done in the stable, a sterile Petri dish, the lower surface of which was smeared with sterilized gum tragacanth, was exposed for about a minute to the stable air, when the cover was replaced and the dish wrapped up in sterilized paper.

All these samples were then taken to the laboratory, where the examination was continued; but, before dealing with these results, we may say a word or two regarding the conditions among the patrons.

CONDITION OF COWS. The cows were in good condition and quite clean; there was scarcely a dirty quarter or udder to be seen. There is very little low-lying or marshy land in the neighborhood of the factory; and consequently, the cows are not soiled with dirt or stagnant water.

CONDITIONS OF STABLES. Speaking generally, we must say that the stables were badly lighted and poorly ventilated. The beam ceilings were usually very dusty, and generally festooned with cobwebs. The floors were fairly clean; but in some, the poultry had soiled the mangers, partitions, and racks to such an extent that a heavy, close smell was noticeable. Such stables gave a very large number of colon bacteria upon the plates exposed in the stable air. In other stables, swine were kept alongside the cows; and, in these cases, a very objectionable smell was present, very injurious to milk; and the bacterial content of the air was usually very large and very undesirable. It is also to be borne in mind that the air was quiet in the stables at the time of my visit. The cattle had been out some hours; and there was no disturbance of litter, etc., such as there would be when the animals were brought into the stable for milking.

A visitor to the factory was at once struck with the color of the cloths; and an examination showed that the color in the cloths was due to a chromogenic mould. This mould was very abundant in the stable air; in fact, we isolated it from 20 stables.

BARN-YARD. On several farms, the cows were milked in the yard, or in the adjacent lane. These yards were usually dry, but soiled with the droppings of cows, which constitute a source of infection, especially if wind is blowing. Milk drawn where these conditions were present contained very large numbers of the colon bacillus, and *B. lactis aerogenes.*

WHERE CANS ARE KEPT. The cans were kept in many different places, in or near the barn-yard, against an out building, under trees, on a milk-stand close to the road, or by the side of the house. In no case was there a properly constructed milk-shed, where the milk could be kept

FIG. 2. On this side of the gate is the barnyard, in which the cows are milked. The can is shown on the right, in a position where the milk can be contaminated with undesirable bacteria from the yard.

and cooled. In several places where the cans were kept on a milk-stand beside the road, a good deal of road-dust was seen in them. Cans full of milk were often left in the barn-yard over night, in a favorable situation for the absorption of odors, as well as bacteria from dried particles of manure or dust which might be floating around (See figures 1, 2, and 3).

CONDITION OF CANS. The cans generally in use hold about 30 gallons. They have concave bottoms and one side seam, which is not always smoothly soldered. In some, however, the bottom is convex; and these latter are very much harder to clean, as the whey is left in a channel around the edge, and dirt there cannot be easily removed by the brush.

Very many milk pails are also very badly constructed with pressed instead of soldered seams and convex bottoms. The cans used by the patrons were usually in good condition as regards rust; but farmers purchasing cans should insist that the seam be soldered so that it presents a smooth surface that can be easily cleaned. In too many cases, the point of a knife can be inserted in the ill-fitting seam, and withdrawn covered with slime or dirt. It is impossible to clean a can thoroughly which has this grave defect.

METHODS OF CAN-WASHING. After the cans are emptied, they are usually scrubbed with warm water. Occasionally soda is used, and at some places, wood ashes, the latter being sometimes mixed with dirt or sand, which is apt to scratch the tin covering and permit the acid whey to act on the iron. After the rinsings are poured out, the can is scalded with hot water, poured from a large kettle around the inside of the can. The can is then rinsed with this water, which is left in the can until it is used, or the rinsing water is poured out. In some cases, *after the scalding*, the can is wiped with a rag.

The objection to the scalding in this manner is that the water is not hot enough. The usual practice is to take the kettleful of water from the house to the place where the cans are washed, and set it on the ground until the preliminary washing and scrubbing is finished. By this time, the water, instead of being at a temperature of 212° F., is about 190° F., and is still further cooled when poured down the sides of the cold can. The final wiping dry with a rag is a bad practice, as the cloth is sure to be contaminated with bacteria, which are thus rubbed over and deposited upon the scalded surface of the can. When the cans are finally scalded, they should be turned bottom up, tipped to one side (facing the sun), and left to dry in the air.

COOLING AND AERATION OF THE MILK. So far as I could ascertain, no form of cooler was in use among the patrons. The usual practice seemed to be "dipping," very often in an impure atmosphere, where bacterial infection was likely to occur. A few patrons used the "Kippen" aerator, but found it unsatisfactory in warm weather. Some kept the milk in pails immersed in a trough of water; but with no covering, except a wire screen to keep cats out. Even for large cans, this was the form of co[illegible]g used; and the milk was thus left exposed to bacterial infection from the air.

Very few patrons know the difference between aeration and cooling, aeration meaning to them, in the majority of cases, cooling as well as exposure to air. Further, some cheesemakers in the past stated that cooling milk injured the cheese, or that it was impossible to make good cheese from cooled milk. This idea, promulgated before the use of acid cultures or starters, still lingers around many factories; and it is quite difficult to convince people that proper cooling is far more important than aeration. Aeration is certainly necessary when the milk is dirty or has absorbed bad odors from being drawn in an impure atmosphere; but most of the better forms of coolers aerate as well as cool.

The situation among the patrons having been thus briefly and generally reviewed, a few words regarding the condition of the factory are necessary.

CONDITIONS AT THE FACTORY. The factory is well built, commodious, clean, and well managed. The floors are of wood in good condition, with cellar underneath with concrete floor, which is, however, somewhat broken. The drainage is fair. There is sufficient fall, and the overflow is carried away by a small creek. The water is of good quality and cold even in summer.

The whey tanks are thoroughly washed out every week; but the drainage from them might be improved, as there is naturally some waste where the cans are filled by the milk drawers from the tanks, and this waste does not run away very readily.

FIG. 3. Illustrating how cans are kept, the barnyard being on the left of the fence. This method is objectionable, because the cans are too close to the yard and are not protected from dust.

The windows and doors were unprotected by fly screens. Consequently flies were very numerous inside; and the infection from this source though not generally taken into account may be very serious. Some flies captured in the making-room were put into a test tube of sterilized water, shaken, and taken out again; and the water, on examination, showed that there were as many as 250 000 gas producing bacteria per fly. Dozens of flies, at the time of my visit, were floating on the milk and whey; and they no doubt contributed a large number of undesirable bacteria to the milk. Fly screens on doors and windows would certainly help to keep out these pests.

The utensils were kept in good condition; and also the vats; but the curd sinks needed airing. It is true that they were well washed; but they needed to be sweetened by being exposed to the air and sunlight; and, as they are not used until about eleven o'clock in the day this could easily have been done.

The cheese cloths were of a peculiar red color, especially where they came in contact with the sink. The color was found to be due to a pigment secreted by a mould, which was quite common in the mixed milk, and which I subsequently found to be very common in dusty stables. The cloths were well cared for,—washed and boiled; but the red color was not removed. They were in fact, dyed with permanent or fast color. Of course, in this case, the maker was not to blame; but the patrons by cleaning and whitewashing their stables, could most probably have prevented the trouble.

Causes of Bitter Milk.

Bitterness in milk is produced by several causes, which may thus be classified:

I. Certain foods, such as, Ragweed, Lupines, etc.

II. Advanced lactation, especially just before a cow becomes "dry".

III. Disease of the udder. In Mastitis (or inflamation of the udder), the composition of the milk is considerably changed; and a bitterness, or more commonly a salty taste, is produced. In most cases, this change is revealed by the appearance of the milk, which becomes a watery, serous-like fluid.

IV. The growth of micro-organisms in the milk.

In the first three, the bitterness may be noticed immediately after milking; but if the bitterness is due to an organism, the bitter taste appears only after some time: and it is with this cause that the present investigation deals.

Those not interested in the biology of the bitter organism will please turn to "Effects on Milk," page 14.

The Biology of the Bitter Organism.

As has already been stated, the organism producing the trouble was a Torula, which was found in nearly every sample of milk examined: and in order definitely to characterise this organism, it will be necessary to give an account of its behaviour under different conditions of food, temperature, etc., information of importance to the bacteriologist, and thus necessarily technical, and of little interest to the maker.

Historical Resumé. In 1860, Pasteur (2) whilst working on the theory of spontaneous generation, noticed that milk which had been boiled did not sour like other milk, but became bitter and contained butyric instead of lactic acid. Further, he noticed that the organisms obtained from the bitter milk were different from those he observed in ordinary sour milk. They were motile, and were at first thought by Pasteur to be infusoria. By the use of a higher temperature he was able to destroy them; and then, step by step, he separated the lactic fermentation from the butyric.

Meissl (3) and Leow (4) somewhat later, noticed a bitterness the same as had been observed by Pasteur.

Duclaux (5) in his work on the ripening of Cantal cheese isolated a bacillus *Tyrothrix geniculatus*, which produced a bitter substance in the presence of oxygen. This organism was probably related to the hay bacillus.

Nägeli (6) in 1879 definitely stated that the bitterness was due to the action of micro-organisms which resisted the boiling temperature.

Liebscher (7) wrote on bitter milk in 1883, and noticed that after some days it putrified and that sulphuretted hydrogen was formed. He suggested the possibility that the change was due to bacteria, probably a form of *Proteus vulgaris*.

Hueppe (8), in his first paper published upon this subject, also definitely stated that the bitter taste of sterilized milk which had been standing some time, was not due to a change in function of the lactic acid bacteria, but that in such milk there were other bacteria which resisted the heat to which the milk was subjected and subsequently produced specific changes in the milk. He referred the bitter taste to *B. butyricus*. This germ, however, is not identical with other butyric acid bacteria found by Van Tieghem, Prasmowski, and others.

Loeffler (9) found in milk four bacteria (*B Liodermis*, *B. Mesentericus vulgatus*, *B. lactic albus*, and *B. butyricus*) which gave to sterile milk a biting and slightly bitter taste. These germs produced peptone and butyric acid.

Kruegger (10) isolated from a sample of bitter milk a species of bacillus very similar to, if not identical with, *Proteus vulgaris*. This germ produced butyric acid, as well as the well known putrefactive fermentation. He deemed the trouble due in some instances to micro-organisms, probably *Proteus*, but gave no account of sterilized milk being made bitter by the growth of this germ in it.

Wiegmann (11) found a bacillus in sweet milk which, when grown in sterilized milk, gave a very strong bitter taste in 24 hours. This organism, even after one month's growth in milk, did not curdle or digest the casein. In old milk cultures, the bitter taste changed to a salty bitter one. Wiegmann questioned the conclusion that butyric acid produced a bitter taste, as this acid has of itself no bitter taste. His bacillus produced no butyric acid, and he thought that the bitterness was due to the decomposition of the albuminoids of the milk.

From a sample of bitter cream, Conn (12) isolated a micrococcus which gave an extremely bitter taste when grown in sterilized milk. This organism curdled milk with a slight acid reaction and subsequently the curd was somewhat dissolved, and the liquid formed was slimy.

When grown in bouillon, the odor, taste, and sliminess resembled that of the bitter milk. The presence of butyric acid was determined in the culture, and butter made from the bitter milk was rancid,—after two days it was "strongly rancid, and the taste strong and burning"

This case of Conn's is noteworthy as being the first example of bitter milk naturally experienced. Previous cases were usually associated with imperfect sterilization of milk, due to the growth of spore-forming organisms.

Wiegmann's germ does not seem to have been of economic importance, as it was isolated from sweet milk, and Kruegger cannot be said to have substantiated his statements by direct infection and subsequent production of bitterness by means of his *Proteus vulgaris* species.

Hueppe (13) in his second paper referred the bitter taste to *Mesentericus vulgatus*, as well as to *B. butyricus*. He expressed the opinion that bitterness was due to the production of peptones, and in support of his opinion, cited the fact that all the bacteria found in bitter milk belonged to the class of peptonizing organisms (those that liquefied gelatine); but he did not deny that there were bacteria which were capable of producing bitter substances of a special nature. We must also remember that there are very many bacteria which liquefy gelatine, but do not produce bitter milk, which latter is by no means a common occurrence.

Bleisch (14) isolated from milk imperfectly sterilized by the Neuhauss-Groniwald-Oehlmann's method a bacillus which produced bitter milk. Peptone was produced. This germ formed spores which resisted a temperature of 100° C. (steam heat) for six hours. It was probably allied to the *B. butyricus* of Hueppe.

De Freudenreich (15) isolated from a bitter cheese a coccus, *M. casei amari*, which produced a bitter taste in both milk and cheese. It produced acid and liquefied gelatine, a rather remarkable combination. Cheese made from milk containing a culture of this organism was bitter. It produced peptone (0.8%); but the bitterness was not entirely due to peptone, as de Freudenreich succeeded in separating bitter substances which did not belong to the peptones.

The same worker also isolated from cream another species, *B. liquefaciens lactis amari*; and fresh cheese inoculated with this germ had a disagreeable, bitter odor. The germ probably belonged to the putrefying organisms.

O'Callaghan (16) found a yeast which gave a sharp, bitter taste to milk, forming alcohol. He stated that it would be injurious to buttermakers, and still more so to cheesemakers, although no experiments were made to support these statements. He named it *Saccharomyces lactis*, but did not describe it; and Beyerinck also gave this name to a yeast he found in Kephir.

Investigations at Guelph.

During the summers of 1899 and 1900, samples of bitter milk were occasionally sent to our laboratory; but last summer the complaints became so frequent that we thought it advisable to make a thorough investigation of the trouble in connection with the factory above referred to.

Torula Amara

Source and Habitat. A torula which gave a bitter taste when grown in sterilized milk was repeatedly isolated from hundreds of samples of cheese and milk, can-washings, whey, etc., which were analyzed.

The well-known fact that yeasts are constantly found on the surface of fruits and leaves—as, for example. *Saccharomyces Comesii*, found by Cavara (17) on the pedicels of Millet; *Saccharomyces Ilicis*, discovered by Grönlund (18) on the fruit of *Ilex aquifolium*; and a yeast present on the fruit of the Mahwa (*Bassia latifolia*), a Sapotaceous tree from the flowers of which a strong alcoholic drink is made—led me to examine the trees and fruit near where the milk cans were kept; and from the leaves of certain species of maple, I isolated a torula identical with the species so frequently found in the milk and can-washings. This torula I have named *Torula amara*.

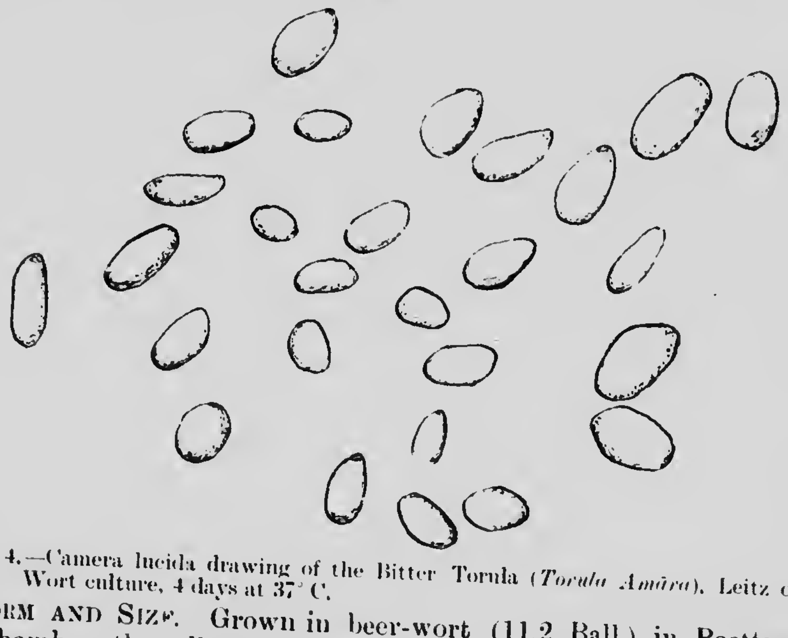


Fig. 4.—Camera lucida drawing of the Bitter Torula (*Torula Amāra*). Leitz oc. 4: obj 1-12. Wort culture, 4 days at 37° C.

Form and Size. Grown in beer-wort (11.2 Ball) in Boettscher's moist chamber, the cells are generally oval in shape, and of varying size. Some are as long as 10 μ, but the average lies between 7.5 and 9.0 μ. After some days' growth, the cells become filled with large vacuoles, and the cell wall somewhat loses its sharp outline. Buds are usually formed at the smaller end of the cell. In milk (24 hrs. at 37° C.) the cells are usually oval, but round and egg-shaped ones occur. The cells occur singly, in short chains of three or four, or in small clumps (See figure 4).

On beer-wort agar at 37° C., the cells are somewhat larger, more irregular in shape, and contain numerous small oil drops.

SPORE FORMATION. No spores are formed. Spore blocks made from young, vigorous cultures, kept at 20°, 25, and 37° C. for 7 days, and observed at short intervals, contained no spores. Cultures in wort, peptone whey, and yeast water never produced spores at the temperatures given above, even when all conditions prescribed by Hansen were presnt.

There has not been any formation of spores in old cultures, some of which have been under observation for four months. Thus the organism is not a true Saccharomycete, but a torula.

WORT (11.2 BALL.) In two days, at 25° C., there is an abundant growth; the liquid is slightly turbid, but there is no pellicle or film On shaking, numerous small bubbles form a deep layer of foam on the surface. In four days the liquid is clear and bright; no pellicle ever forms; about 2 per cent of alcohol appears. In cultures kept in small Erlenmeyer flasks at 37° C., a "yeast ring" forms; but, at room temperature, no ring develops, even after two months' growth.

The reaction of the wort, after fermentation ceases, and the carbonic acid gas which is driven off, is very slightly acid. In whey containing 1 per cent. peptone and .25 per cent Nährstoff Heyden, the growth is very similar to that in wort. There is an active fermentation and the reaction of the medium afterwards is slightly acid. In whey which has been boiled or filtered, 3 per cent alcohol forms in 8 days at 37° C., and no trace of sugar is found. In yeast water, there is a heavy deposit; the body of the liquid is clear and bright, and no film is formed.

WORT GELATINE. In two days, at 20°, pin point colonies, about 0.5 m. m. in diameter, are visible, the surface ones being slightly convex. Under the microscope (2-3 obj.), the colonies are dark with entire edges. In four days, thev are round, greyish white, shiny, and from convex to capitate. They are not at all characteristic.

BEEF PEPTONE GELATINE. Colonies in this medium are somewhat smaller, but resemble those in wort gelatine. In four days, they are still similar to those described above, but under the microscope the edge of the colony is slightly rough.

WORT GELATINE STICH. In ten or twelve days, the line of puncture is beaded. The beads are distinct, and of considerable size; later (20 to 30 days), a dense, spiny growth radiates from the beads; but the projections are not sufficiently long or branched to justify the term arborescent. They may be called echinate. The surface growth spreads from 10 to 15 m.m., is umbonate, waxy, greyish white in color, but of a light brown shade towards the centre.

WORT GELATINE MASS CULTURE. These cultures were made by placing a drop of wort culture on the surface of the gelatine. The surface growth spreads over the entire surface in 30 days. The edges are thin and slightly rough. The centre is elevated, forming a plateau, which is quite flat, or slightly depressed, and about 8 to 10 m.m. in diameter. The under surface of the growth is pulvinate and extends downward into the gelatine to a depth of 4 m.m.; and very short, crowded, echinate projections demarcate the line of contact between the growth and the gelatine.

WORT AGAR SLOPE. Grows very quickly at 37° C., and in three days a slightly raised, spreading, greyish, white, moist, glistening growth is seen, which becomes more massive with age.

BEEF PEPTONE AGAR SLOPE. In eight days, at 25° C., there is a flat, somewhat glistening growth, iridiscent like mother of pearl.

POTATO. On this medium there is a slightly raised growth in three days at 20° C., only distinguishable from the color of the potato by its dull appearance. In seven days there is no change. At 37° C., the growth is slightly yellower, otherwise similar to that at 25° C.

SACCHAROSE MEDIA. In Conn's medium, with the addition of 6 per cent. saccharose, a trace of gas forms after seven days' growth at 25° C. No film is formed, but there is a heavy deposit.

In yeast water, with the addition of six per cent saccharose, and under the same conditions, considerable gas is produced. There is no film, the body of the liquid in both cases being clear; but there is a heavy deposit. The sugar is inverted (Fehling's solution) In bouillon containing one per cent. peptone, 25 per cent. Nahrstoff Heyden, five per cent. salt, and 6 per cent. saccharose, gas is produced, and the growth resembles that in saccharose yeast-water.

DEXTROSE MEDIA. In Conn's medium, with six per cent. glucose, there is a slight trace of gas in seven days at 25°. There is no film, no turbidity, but a heavy deposit.

In yeast water, dextrose (six per cent.), the growth is similar to that Conn's medium, but there is more gas

In peptone-Heyden-saccharose (six per cent.) bouillon, some gas is produced, but otherwise the growth is the same as above.

LACTOSE MEDIA. In Conn's medium, with six per cent. lactose, there is considerable turbidity in both arms of the tube. There is no film, and 90 per cent. gas is formed.

In yeast-water lactose (six per cent.), the liquid is clear, there is no pellicle and 95 per cent. gas is formed.

In peptone-Heyden-lactose (six per cent.) bouillo he growth is similar to that in yeast-water lactose, and 96 per cent. gas is produced.

MANNITE MEDIA. Conn's medium, with six per cent. mannite, is not fermented, and there is but slight growth.

In yeast-water mannite no gas is formed, but the growth is slightly heavier than in Conn's medium.

MALTOSE MEDIA. In peptone-Heyden-lactose (six per cent.) bouillon, a slight trace of gas is formed. There is no film, the liquid is clear, but there is a heavy deposit.

Effects on Milk.

Milk. In sterile milk the bitter taste can be detected after five or six hours growth at 37° C. In 14 hours the taste and smell are disagreeable and strong. The odor of cultures a few days old resembles plum kernels, and the taste is very unpleasant, bitter and astringent. In 10 days, at 37° C., the milk is slightly thickened, slightly acid, and the odour and taste are both pronounced. A slight, aromatic, ethereal smell may also be detected, but it is frequently masked by the bitter taste. There is much gas formed.

Culture gives the biuret action, indicating the presence of peptones. No butyric acid is present.

Effects of Drying, Antiseptics, etc., on Torula Amara.

Thermal Relations. The optimum for growth is about 37° C. (98.6° F.). Growth at 25° C. is abundant, and at 20° considerably slower. No growth occurs at 5° C. (41° F.), and none at 48° C. (118.4° F.)

The thermal death point was determined in triplicate, by Sternberg's method in whey bouillon:

Ten minutes at 56° C.: living.

Ten minutes at 60° C.: in two tubes, no growth; in one, much growth.

Ten minutes at 62° C.: no growth, all dead.

A moment's exposure at boiling temperature (98.5° C. in Guelph): growth.

Five seconds at boiling temperature: no growth.

Ten seconds at boiling temperature: no growth.

As this Torula was found in the can washings of nearly all the patrons, a practical test was made to see if the ordinary scalding with water at about 200° F. was sufficient to kill it. A 25-gallon, straight-sided can was thoroughly steamed, and then rinsed with a culture of the bitter torula. At the end of twelve hours the can was cleaned with warm water and a kettleful of water at 200° F. poured down the inside and then well rinsed, and the water poured out. After this, the can was rinsed with sterile wort, which was then collected in a sterile bottle, and incubated. In 12 hours there was abundant development of the Torula. The amount of water used for scalding was 3 pints; and the experiment was afterwards repeated using 12 pints, but with similar results, the Torula not being killed.

Effect of Desiccation. Cover glasses spread with a 24-hour-old culture were exposed in the open air to dry at a temperature which varied from —15° C. to 5° C. A cover glass was placed in whey bouillon at the end of every 24 hours. For the first 7 days, growth occurred in all the cultures; but on the 8th and following days no growth occurred.

Effects of Antiseptics. A 2 per cent. soda solution at 55° C.

(131° F.) failed to kill the Torula in 5 minutes, but in 10 minutes all the cultures were sterile. A 4 per cent. solution killed the Torula in 5 minutes.

ACTION OF ACIDS. The determination of the amount of acidity in the culture medium (yeast water) was made after sterilization.

(1) Tartaric acid (1.125 per cent.)—More growth occurs in cultures with this amount of acidity than in the control cultures.

(2) Tartaric acid (2.025 per cent.)—There is some growth, slightly less than that in the control tubes.

(3) Lactic Acid (1.2 per cent.)—More growth occurs in cultures with this amount of acidity than in the control tubes.

(4) Lactic acid (2.4 per cent.)—There is a flocculent deposit, and less growth than in the control tubes.

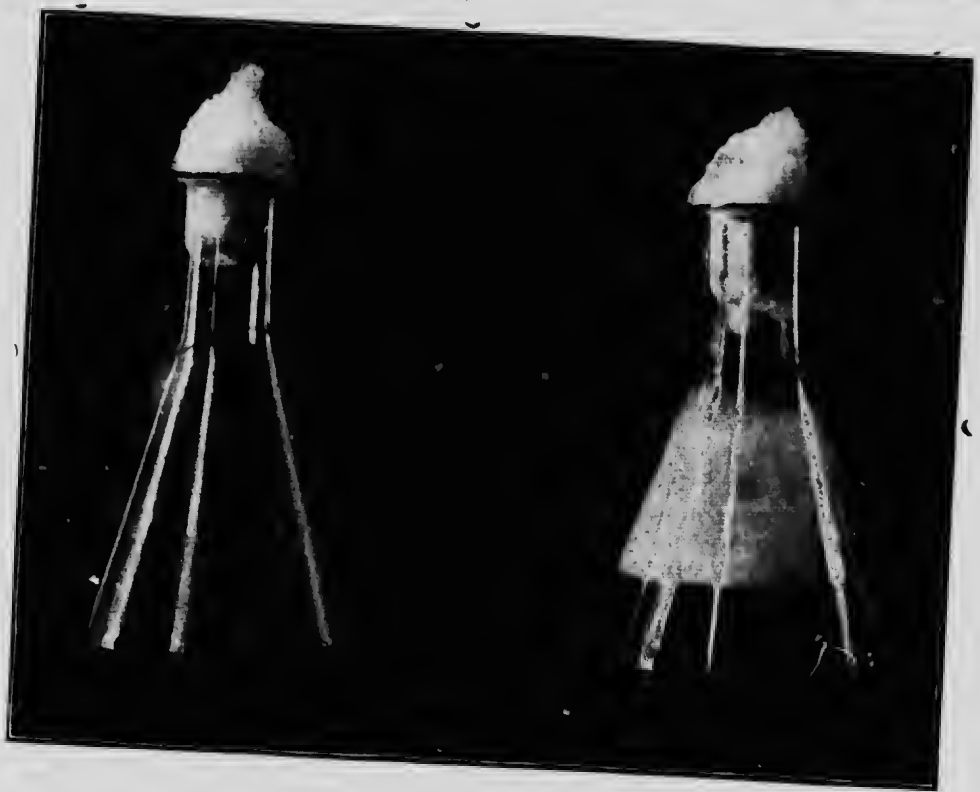


Fig. 5. The bitter Torula (*torula amara*) in whey—(*a*) before shaking; (*b*) after shaking. Note the large amount of gas (froth) in the latter.

EFFECTS OF ACID WHEY ON TORULA AMARA.

By the rapid development of acid in whey, cheesemakers are able to control many abnormal fermentations; and it is, therefore, of considerable importance to ascertain whether or not the bitter flavor may be controlled in this way A number of experiments were made to determine the effect of acidity on the bitter Torula.

In the first series, acid whey, containing 1.08 per cent. lactic acid, was used, and the vigor of growth was determined by the amount of fermentation. In such a medium, 50 per cent. gas was formed. The control culture in ordinary whey gave 85 per cent. When neutral sterile whey was inoculated with the bitter Torula and B. *acidi lactici* together, 70 per cent. gas was formed. Acid whey (1.08 per cent.) simultaneously inoculated with the Torula and the lactic acid bacillus gave 48 per cent. gas.

These results, taken alone, seem to indicate that a considerable amount of acidity somewhat prevents the activity of the Torula ; but I subsequently repeated the above experiments, using the same Torula ; and, after repeated transfers in whey, it gave a more vigorous fermentation than originally. With this culture in fermentation tubes containing whey peptone bouillon, kept at 37° C. for 48 hours, I obtained the following results :—

Torula amara	90	per cent.	gas.
" "	88	"	"
" and lactic acid bacillus	90	"	"
" " "	76	"	"
" " "	87	"	"

Whey and skim-milk mixed :—

Torula	93	"	"
Torula and lactic acid bacillus	94	"	"

Skim-Milk. In this case, the lactic acid bacillus coagulated the milk and formed a solid curd, which shrunk and adhered to the sides of the tubes ; but the gas forced the whey into the open arms, leaving the following amounts of gas in the closed arm :

Torula	94	per cent.
Torula and lactic acid bacillus	92	" "
" " " " "	89	" "

These more extended experiments go to show that the development of acid does not hinder the growth of the Torula ; and the results are in accord with what we know of other yeasts. For example, an acidification with lactic acid bacteria is used in distilleries to prevent the growth of injurious bacteria ; but the amount of acidity present in the mash, about 1.1 per cent., does not prevent the *subsequent* growth of the yeast.

A final experiment on the influence of acid and temperature on this Torula was made by growing the Torula in whey containing 1 per cent.

lactic acid, at temperatures of 20°, 25°, and 37° C.

Average of Two Tests.	20° C.	25° C.	37° C.
Original number in flask per c. c	3	1	1
Six hours after	5	4	72
Nine hours after	5	15	350
Twelve hours after	93	290	2050

With the optimum (or best) temperature (37° C. and 98.6° F.), the growth was very rapid, and fully accounts for the strong development of the bitter flavor under factory conditions.

We conclude, then, that the development of acid in milk or curd, whether by the regular process of souring, the addition of a starter, or inoculation with the lactic acid bacillus, does not destroy the torula, nor remove its injurious effects. (The non-scientific reader will please turn to page 18.)

In the above account of the Torula amara, I have emphasized the economic importance of the organism, and the injury it causes when present in cheese; but it has a very vigorous action on milk sugar also; and organisms possessing this power are not very common, especially if we exclude the yeasts which have been described as occurring in Koumiss and Kephir granules. Pirotta and Riboni (19) in 1879 described a yeast which fermented milk sugar, and named it *Sach. galactocola*; but the description, in accordance with the methods then in use, is not sufficiently exact to enable us to identify the species.

Duclaux (20) obtained a lactose fermenting yeast from milk which was fermented. This species converted all the lactose present into alcohol and did not coagulate the milk but made it viscous, and had a strong anaerobic tendency. Duclaux was unable to isolate an enzyme, and suggested that the yeast acted directly through its growth, and not by the secretion of an enzyme.

Adametz (21) described under the name *Sach. lactis*, a species which he thought different from Duclaux's (22); but the latter author was rather sceptical about its being a new species, and thought it was the same one as he had found.

Subsequently, Kaiser (23) having found a lactose fermenting yeast, made a comparative study of the three varieties, and came to the conclusion that his species was intermediate between the species described by Duclaux and Adametz.

Grotenfelt (24) and Wiegmann (25) also described lactose fermenting yeast, but they did not describe them sufficiently for subsequent recognition.

Beyerinck (26) in 1899 isolated two yeasts, one from Kephir, which he named *Sach. Kefir*, and one from Edam cheese, *Sach Tyriocola* which fermented milk sugar and produced alcohol; and he further isolated from them an invertive enzyme which he called "lactase", which he found had the power of inverting the milk sugar, and cane sugar but not maltose.

This conclusion was, however, contradicted by Schnurmans-Stekhoven (27), who was unable to invert milk sugar with the enzyme "lactase"; and afterwards de Freudenreich (28) came to the same conclusion.

Bochiocchio (29) has also isolated a top fermenting yeast from Grana cheese, which produces blisters on the cheese. This form coagulates milk with partial peptonization, and produces an agreeable foaming beverage when grown in whey.

Jorgensen (30) isolated a true Saccharomycete from Kephir, which produces about 1 per cent. of alcohol in the course of 8 days. This species he named *Sach. fragilis*, on account of the feeble powers of resistance of the cell wall. It produces oval spores, which are soon set free. So far as I am aware, this is the only species of lactose fermenting yeast yet discovered which is a true Saccharomycete.

Duclaux (31) who reviewed the lactose fermenting yeasts in 1900, came to the conclusion that the only three yeasts which fermented sugar (milk sugar) were those isolated by himself, Adametz, and Kaiser. Those subsequently isolated are either insufficiently described, and consequently one cannot say whether they are new species or not; or else they simply assimilate the lactose and burn it in contact with the air.

The *Torula amara* ferments milk perfectly, even when oxygen is not present. Cultures were grown in whey bouillon in an atmosphere of hydrogen, and the sugar of the medium was fermented. In ordinary whey cultures, the sugar is completely fermented, as the following experiment shows:

Whey containing 37.65 grams of sugar per litre before fermentation, gave no trace of sugar (Fehling's solution) after eight days' growth; and the fermented solution was found to contain nearly 3 per cent of alcohol.

Kaiser suggested that certain of the lactose fermenting yeasts might be of economic importance; and on account of the complete fermentation of sugar by the *Torula amara*, it might, we presume, be used in the manufacture of alcohol from whey.

Bitter Cheese Experimentally Produced.

A peculiarity about some of the bacteria that produce bitter milk is that the bitter taste disappears when the milk is made into cheese; but the *Torula amara* produces bitter milk and bitter cheese also. We made several cheese, using cultures of the Torula, and the cheese thus made were very bitter.

The cultures used in the first experiments were made by breaking up a small piece of cheese in sterilized milk, and leaving this for 24 hours at a temperature of about 85° C. The cultures thus secured naturally contained a variety of micro organisms, probably in somewhat the same ratio to one another as would be met with at the factory. Two pounds of this culture was added to 115 pounds of milk, and left in it for 65 minutes before setting.

The [illegible]d developed slowly before [illegible]ipping, but afterwards worked very fast, and had more than the usual amount of acid present at times of milling and salting—0.9 per cent. and 1.143 per cent. acid respectively. The curd was somewhat gassy, and the flavor bad, both at the time of making and subsequently, when the cheese was scored by competent judges. The total score was 76 per cent., flavor receiving only 25 points out of 40.

Subsequently several cheeses were made with p[illegible]ultures infected by the bitter Torula. In these cases, the flavor was not absolutely identical with that of the cheese from the factory referred to; but this is not to be wondered at when we remember that the ratio of the organisms to one another in the experiments was not identical with the ratio under the ordinary factory conditions; but, in every case, the product made was bitter and disagreeable, and the aromatic flavor noticeable on the vats of the factory was always present to a greater or less extent.

The scoring of these cheese was as follows:

	Max 100.		Max. 40.
Cheese No. 1, total score....	59.	Points for flavor....	15
" " 2, " "	74.	" "	22
" " 3, " "	76.	" "	24
" " 4, " "	66.	" "	20

Bacteriological Data.

A large number of analyses of experimental cheese were made, and the results from all of them were fairly constant; as a sample of these results the following quantitative analyses will serve:

Cheese made September 24th, 1901, two per cent. of a culture of the bitter organism used.

Age of Cheese.	Lactic Acid Bacteria. Per gram.	Bitter Torula. Per gram.
7 days	150,000,000	72,000,000
28 "	28,000,000	19,000,000
42 "	11,500,000	3,200,000
71 "	864,000	388,000

The judges reported this cheese to be very bitter and undesirable. Score for flavor, 22 out of 40.

Cheese made [illegible] 12th, 1901, two per cent. of a culture of the bitter organism us[illegible]ry little acid developed during the making, but

.495 per cent. was present at time of milling A strong bitter flavor and aromatic "apple pomace" odor were perceptible at the time of making.

Age of Cheese.	Lactic Acid Bacteria. Per gram.	Bitter Torula. Per gram.
7 days	119,000,000	85,000,000
20 "	55,000,000	7,500,000
30 "	51,000,000	3,800,000
42 "	43,000,000	1,665,000

Score for flavor, 15 out of 40. Total score, 59 per cent

In order to study the cheese made at the factory, three were brought to Guelph, one of which was placed in cold-storage at an average temperature of 39° F., and the other two were put into the ordinary curing-room. The cheese exhibited a fair sample of the trouble complained of at the factory,—they were quite bitter.

Cold Storage Cheese :

Age.	Lactic Acid Bacteria. Per gram	Bitter Torula. Per gram.
28 days.	342,000,000	3,200,000
42 "	271,000,000	[illegible]70,000
90 "	48,600,000	162,000

Left in the ordinary curing-room :

No. of Days.	Lactic Acid Bacteria. Per gram	Bitter Torula. Per gram.
28	59,000,[illegible]	960,000
Oct. 21. 42	38,400,000	800,000
Dec. 4. 90	5,184,000	58,000

The judges considered that the refrigerator cheese was superior in flavor to the cheese in the ordinary curing-room, although both were made from the same vat.

The bacteriological results show that a larger number of yeasts were present in the cold-storage cheese, and the number of acid bacteria present was also greater. When we take into account the proportion between the acid forms and the yeast, we notice that in the cold-storage cheese the ratio at 42 days was 1:147, and in the ordinary curing-room, 1:48. At 90 days, it was 1:300 and 1:90, respectively. Thus the proportion of bitter yeast to lactic acid bacteria was much greater in the cheese kept in the ordinary curing-room; and this may possibly account for the difference in the flavor noticed by the judges, although the refrigerator cheese could not be called good, as the bitter flavor was quite noticeable.

To some, however, it may seem that the number and proportion of the bitter torula to the lactic acid bacteria was out of all proportion to the effect produced; in other words, that there were too few of the bitter torula to produce so much bitterness and bad flavor in the cheese. We must remember, however, that the torula is fully 20 times as large as the lactic acid bacterium; and we may assume that the changes brought about by the torula were in proportion to its size.

Several analyses of curd taken from the factory, just before putting to press, gave as many as 21,000,000 torula per gram; and taking into account the comparatively large size of the organism we see that these results are quite in accord with the bad flavor produced in the cheese.

Butter Experiments.

Professor Dean suggested that if the factory was turned into a creamery for a season or two, the bitter flavor might disappear; but before suggesting that course, we thought it advisable to conduct a few experiments to determine the action of the bitter torula on cream, and its subsequent action on butter.

The experiments on the effect of the bitter torula were made with both sterilized and pasteurized cream.

Sterilized Cream. The lot of sterilized cream was ripened with a starter composed of a culture of lactic acid bacteria and a culture of the bitter torula in equal proportions; 15 per cent. of this mixed culture was used, and the cream was ripened at 22° C. (70° F.) for 18 hours. The acidity at churning time was 0.72 per cent., and the taste was sour and bitter. The cream was cooled and churned. The butter in the granular stage was of good appearance; but somewhat spongy. Two days after making, the sample was scored by an expert judge, who gave it 32 points out of 45 for flavor; and he remarked that the butter had a flat, tallowy taste, without butter aroma.

Pasteurized Cream. The lot of pasteurized cream (160° F.) was ripened with a starter containing the bitter torula, for 12 hours, at 68° F. Very slight acidity developed, but the sample churned easily in 18 minutes. The granular butter was very pale in appearance, had an aromatic odor, and a nauseating, bitter, astringent taste. It would be impossible to sell such butter in any market.

Subsequently several churnings were made as detailed above; and in every case the butter was very bad, and had a pronounced, bitter, disagreeable taste.

These experiments show that the trouble cannot be got rid of by changing from cheese making to butter making for a time.

The majority of summer creameries in this Province do not pasteurize their milk; and where pasteurization is not done, there is danger of trouble from bitter organism, especially if the skim-milk is returned to patrons in the cans in which the whole milk is conveyed to the factory; and the danger is the same whether the factory is used for making butter or cheese.

Summary

Of the bacteriological analyses of stable air, fore-milk, can washings, and mixed milk of 96 patrons of the K—— factory.

T, Bitter Torula ; L, Lactic acid bacillus ; C, Bacillus coli ; A, Bacillus lactis aerogenes ; S, The hay bacillus ; O, other yeasts than the bitter one Y, Ropy milk bacillus ; M, Moulds ; R, Red mould ; X, Harmless bacteria ; P, Proteus group.

No.	Stable, clean, + ; dirty, —; ventilation, etc.	Germ content of stable air.	Germ content of fore-milk.	Condition of cans, where kept, etc.	Germ content of can-washings.	Germ content of mixed milk.
1	+	M – only a few	L	good, left long before washing	T, O, G, etc	T, O, A, G, L, etc.
2	—	M, R, etc	L	near fruit trees	T, O, A, & C, etc	T & O (1,000,000 per c.c.) S. A. C.
3	—	M, R, C, etc.	L & X	" "	T, O, S.	T & O (8,000,000 per c.c.) C. A.
4	—	M, R, P, & C.	L & X	old and rusty, trees	T, C, S, etc	T, 125,000, A, 50,000.
5	— and badly lighted	M, R, C, & Y	L	near barnyard	O, Y, etc	T, O, C, A, 15,000 S, etc.
6	+	M & C.	L & X	under apple trees	T, O, etc	T, O, A, 150,000, S.
7	— and very dark	M, C, & P	L, etc.	under fruit trees	T, O, S, etc	T, O, C, 100,000 & A, 200,000.
8	— and dark	M, 5 species, R & C.	L & X	under apple tree	T, O, etc	T, O, A, 300 000, P & R.
9	Milked in rd which was dusty			under hops and near barnyard	T, O, S, etc	T, O, A, C, S, etc.
10	—, +	M & C	L	old, rusty, and near trees	T, O, etc	T, O, C, A, etc.
11	—	M, R, C, & S.	L, etc.	under fruit trees	T, O, S, etc	T, O, A, etc.
12	—	M, R, C, & P.		close to barnyard	T, O, etc	T, 600,000, C, A, etc.
13	Milked in yard		L, etc.	close to road and pig-pen	T, S, C. etc	T, A, 125,000, C, 5,000,000, etc.
14	—	M & C.	L & X	near fruit trees	T, O, S, C, etc	T, 1,000,000, O, A, 600,000 & C.
15	+ ; —	M, C, & O	L	under maple tree	T, O, S, etc	T, O, C, A, & R.
16	—	M & C	L & X	" "	T, S, A, etc	T, 2,000,000, O, A, 400,000 & C.
17	Milked in yard			near barnyard	T, O, etc	T, O, C, & A.
18	—	C & P		near barnyard and trees	T, O, very few bacteria	T, O, C. A, S, etc.
19	Milked in yard		L, etc.	near apple trees	T, O, etc	T, O, 200,000, S, etc.
20	—	M, R, [illegible]		in barnyard	T, S, etc	T, 400,000, etc.
21	Milked in orchard		L	under fruit trees	T, & O, enormous Nos	T & O, 1,400.000, & C.
22	+	M. P, few	L & X	by roadside	T, O, etc	T, O. 100,000, & C.
23	—	M, C, & P	L, etc.	"	T, O, etc	T, O, 400,000, A, C, etc.
24	—	M & P		in barnyard	T, O, etc	T, O, C, S, & R.
25	—	M, C, & P		near barnyard, under maples	T, O, many S	T, O, C, & A, 100,000.
26	Milked in yard			under fruit trees	T, O. etc	T, O, C, & A.
27	—; +	M, R, & P		under trees	T, O, etc	T, O, 3 species, C, & A.
28	—	M, C, & P		in barnyard	S, C, etc	S, O, etc.
29	—	M & C		under trees	T, O, etc	T, O, C, etc.

30	—, chicken dirt	M & C, very many		under maple tree	T, O, S, etc	
31	— and dark	M, R, C, & P.		under maple and poplar trees	T, O, S, C, etc	T, & O, 100,000, C & P.
32	Milked in yard			near trees	T, O, S, etc	T, & O, 100,000, C & A.
33	—	M, R, C, & P		near barn	T, Y, S, etc	T, 50,000, C, 100,000, A, etc.
34	Milked in yard			under maple tree	T, etc	T, 5,000, C & A, 400,000.
35	Milked in yard			" "	T, etc	T, A, & S.
36	—	M, R, & C		under fruit trees	T, O, etc	T, C, & A.
37	—	M & C		under fruit or maple	T, O, S, etc	T, O, C, A, S, & R.
38	—	M & C		under trees	T, very few, etc	T, O, A, C, etc.
39	Milked in lane		L	stand under maple	T, O, very numerous	T, A, S, P, & R.
40	—; +	M. R, & C		" "	T, O, very numerous	T, O, A, & P.
41	—; +	M. & P		under fruit trees	T, very few, S, & Y	T, O, A, C, & R.
42	—	M, C, P, etc		" "	T, O, etc	T, O, A, & R.
43	— and ventilation bad	M, C, & R		bad cans, under maple trees	T, O, numerous	T, O, C, & A.
44	—; +	M, C, & P		near a vine	T, O, & S	T, O, A, S, & R.
45	Milked in yard			under fruit trees	T, O, S, etc.	T, O, S, & A, 100,000.
46	—	M, R, & C		cans dirty	T, O, S, & P	T, & O, 1,500,000, & A.
47	— and ventilation bad	M & C		under vine and maple	T, O, etc	T, & O, 2,500,000, A, & C.
48	—	M & C		left long without washing	T, & S, very numerous	T, & C, 1,600,000, etc.
49	—	M		in a wooden trough	T, S, etc	T, 400,000, S, A, etc.
50	—; +	M & C		under trees	T, O, S, etc	T, O, S, C, & R.
51	Milked in lane			near vine	T, O, etc	T, O, S, C. & R.
52	—	P & C.		near fruit trees	T, O, etc	T, & O, 400.000, C, & S.
53	Milked in lane			cans dusty	T, O, S, etc	T, & O, 2,000,000, C, & S.
54	Milked in lane			left long without washing	T, O, etc	T, O, 3 species, C, A, & S.
55	+	M & C		dirty cans	T, O, etc	T, O, C, A, etc.
56	—	M, C, & P		near stable	T, O, etc. Pails, only bacteria	T, O, C, A, etc.
57	—	M, C, & P		left long without washing	T, M, etc	T, 50,000, C, 100,000.
58	+	M & C		left lying on ground	T, etc	T, O, C, & A, 290,000.
59	Smell from piggery	M & C		near fruit trees	T, O, S, etc., also pails	T, C, & A, 150,000.
60	—	M		near the lane	T, S, pails, S, etc	T, O, C, A, & R.
61	—	M & C		under an apple tree	T, etc., pails too	T, O, C, & A.
62	—	M		under poplars	T, S, etc	T, & O, 2,500,000, C, & A.
63	Smell from swine	M, R, C, & P.		under vine	T, C, S, etc	T, & O, 1,500,000, C, & A.
64	+	M & C		cans contained rain water	T, O, etc., pails, no yeasts	T, C, P, & S.
65	—, and ventilation bad	M, C, & P, numerous		under maple trees	T, O, etc	T, C, & A.
66	Milked in lane.			under poplar	T, O, pails, T and S	T, C, A, P, & R.
67	—			milk sieve dirty	T, S, etc	T, A, C, 250,000, etc.
68	+; —	M, R, P, etc.		kept in trough	T, S, etc	T, 5,000, C, & A, 1,200,000.
69	—, and ventilation bad	C & M		left long before washing	T & O	T, 300,000, & etc.
70	—	M & C		lying in grass	T, etc	T, A, & P.
71	—	M, R, P		not clean, near trees	T, O, S, P, & C	T, O, & A.
72	—; +	M, R, & C		under fruit, near stable	T, O, etc	E, S, C, A, etc.
73	Milked in lane	M, P, & C		under fruit trees	T, O, etc	T, O, 2,200,000, C, A, etc. T, O, C.

Summary

Of the bacteriological analyses of stable air, fore-milk, can washings, and mixed milk, etc.—*Continued.*

T, Bitter Tornla : L, Lactic acid bacillus ; C, Bacillus coli ; A, Bacillus lactis aerogenes ; S, The hay bacillus ; O, Other yeasts than the bitter one ; Y, Ropy milk bacillus ; M, Monlds ; R, Red mould ; X, Harmless bacteria ; P, Protens group.

No.	Stable, clean, + ; dirty, — ; ventilation, etc.	Germ content of stable air.	Germ content of fore-milk.	Condition of cans, where kept, etc.	Germ content of can-washings.	Germ content of mixed milk.
74	—	M, P, & C		under poplar trees	T, etc	T, O, C, S, etc.
75	+	C, few		in the lane	T, O, S, etc	T, O, C, A, & P.
76	+; —	M, P, etc		under maple trees	T, etc	T, C, & R.
77	—	M, C, & P		on grass	T, S, etc	T, O, A, & C.
78	Ventilation bad	M, C, & P		on the milk stand	T, O, S, etc	T, A, C, & R.
79	– ; +	M, R, & C		cans dnsty, under maple	T, O, etc	T, O, A, & C.
80	+	M		between house and stable	T, O, S, C, etc	T, O, A, C, etc.
81	+; —	M & P		nnder basswood		

In the cases of the following patrons, the mixed milk only was examined.

81 The bitter torula, another yeast and B. coli.
82 " " " "
83 " " two yeasts and Red mould.
84 " " B. coli and a capsulated bacillus.
85 " " B. subtilis, and B. coli.
86 " " Oidium lactis, B. subtilis, and a capsule bacillus.
87 Two species of yeast, B. lactis aerogenes, and Red monld.
88
89
90
91
92 } The milk from all these patrons contained one or two species of yeasts, B. coli, or B. lactis aerogenes, or both were present in all samples.
93
94
95
96

The above tables summarize as briefly as possible the results of personal visits to the patrons of the factory, and the bacteriological analyses of fore-milk, stable air, can washings, and the mixed milk of each herd.

I regret to say that the stables were very generally dirty, the beams and ceilings being covered with dust and cobwebs; and in a number of cases, the floors, stanchions, etc. were fouled with the droppings of cattle or poultry. In such cases, the stable air gave large numbers of *B. coli* and *B. lactis aerogenes*. In such stables, moulds were usually present in large numbers; and the Red Mould, of which mention has already been made, was found in 19 stables. All the micro-organisms isolated from these dirty stable airs were unfavorable to cheese and butter making. In cases where milking was done in the barn-yard, there usually was a large infection from the Colon bacillus and other undesirable species.

Only a limited number of analyses of the fore-milk were made, as the bacterial content did not seem to warrant this line of investigation. The *B. acidi lactici* (Esten) and some slow growing harmless bacteria were isolated from the fore-milk or first milkings of about a 100 cows. In no case was the Bitter Torula present nor any representatives of the Colon group.

In view of the large sums of money which patrons receive from the factory, it is astonishing that little or no effect has been made to provide proper places for keeping the milk. The only general precaution taken is to cover the can with a wire screen to keep out the cats! and in many cases the place selected for the can is most unsuitable. The patrons, as a rule, seem quite unaware of the possibilities of infection from road-dust and barnyard air; and they do not understand the importance of controlling the temperature.

The huge numbers bacteria and the great diversity of species found in the can washings were very noticeable throughout our investigations. The Bitter Torula was present in all samples but two; and in one of these, the farmer did not draw the whey home in his milk can but used another vessel. I have elsewhere dwelt on the difficulty of washing the cans properly on the farm; and from the bacteriological evidence here presented, we are quite safe in saying that when the milk cans are once thoroughly infected with micro-organisms, it is almost impossible to kill these organisms by the methods of can washing generally practised on the farms of this Province. We should not, however, lose sight of the fact that the more thorough the washing, the less the liability of infection from undesirable bacteria.

The mixed milk of the different patrons contained the bitter organisms in varying numbers. In some, very few torulae were present; in others, they existed in enormous numbers. In samples of whey taken from the vats, there were large numbers; and it is evident that these organisms carried back in the whey became a permanent source of infection. They may in fact be described as endemic (produced by or depending upon special local conditions) in this district. Hence, even if the cans are washed and sterilized, there may occur conditions which will produce a recurrence of the trouble.

The cheesemaker of the neighboring factory (who washes and sterilizes the milk cans in the factory) informed me that on one or two, occasions during the summer bitter milk was delivered at his factory; and he added "we should undoubtedly have had serious trouble, had it not been forthe fact that we wash and *sterilize* all milk cans in the factory."

In addition to the bitter torula, the Colon group of bacteria were well represented; and they are most undesirable for the maker, as they give rise to gas and disagreeable flavors, and in colored cheese produce mottles. Certain bacteria belonging to the *B. lactis aerogenes* group also produce a somewhat bitter taste in milk; and these add slightly to the bitterness produced by the *Torula amara.*

A bacillus producing slimy milk was also occasionally found in the can washings and stable air, a fact which shows that bacteria of this class are rather wide-spread; and, given certain conditions, an epidemic of slimy milk may occur. Nothing more concerning this germ need be said in this connection, as the habitat and characteristics of slime-producing bacteria will be the subject of a future publication.

Remedial Measures.

The inquiry into the condition of the stables, cans, pails, etc. of the patrons of the K—— factory together with the bacteriological evidence above related, show that certain measures should be taken in order to remedy the present condition of affairs.

The dominant note sounded at the recent Dairymen's Convention was *better care of milk*; for unless this care is given, it is impossible for the butter or cheese-maker to produce a prime article. According to many speakers and writers, the time has come when contracts should be made between the milk producer and the factory executive; and these contracts should contain provisions for the exclusion of fowl and swine from cow stables, the care of stables so as to avoid the accumulation of dust, cobwebs, etc., the whitewashing of stables twice a year, cleanliness, in milking, efficient aeration and cooling, proper places for keeping the milk, the delivery of the milk at the factory at a certain temperature, and the proper washing of utensils. Should these improvements be made, I am sure that the results would surprise all concerned; and the little extra care involved would be amply repaid by the higher price of cheese made from milk handled in a careful, sanitary manner; for it is well known that buyers discriminate and place considerable emphasis on the reputation of a factory; and they would, we think, quickly notice the merits of such a system as that suggested.

In order to get rid of the bitter Torula at the K—— factory, and improve the general conditions under which the milk is produced, m sures should be taken to remedy defects as regards—

1. The stables.
2. The cans, pails, and utensils.
3. The control of the temperature.
4. The places in which the cans are kept before and after the milk is put into them.

1. THE STABLES. The bacteria existing in the air of most of the stables are very undesirable, especially those whose habitat is in the manure, dried particles of which are wafted about in the building by the slightest currents of air and fall into the milk pails during milking, and during straining when the cans are kept in the stable or close to the barn-yard.

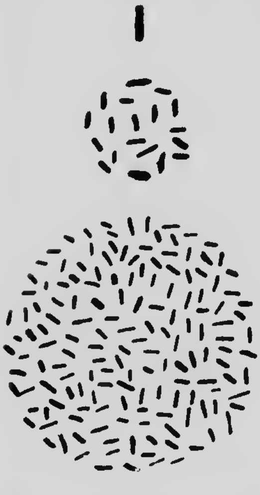
Stables should be kept clean (as free as possible from cobwebs and accumulations of dust), and be whitewashed twice a year, once in the fall and again in the spring when the cows are put out to pasture. The white-wash should be made from fresh lime; and after slaking, a wash of about the consistency of cream should be made, strained through a piece of sack-ing, and applied by means of a brush or ordinary spray-pump. In the latter case, two or more applications are necessary each time. A little molasses, size, or Portland cement added to the whitewash increases the adhesiveness. In case disinfection is thought to be necessary, crude car-bolic acid may be used in the proportion of a pint to every gallon of whitewash.

Whitewashing not only decreases the bacterial infection; but it in-creases the amount of light in stables, thus directly promoting the health of the stock.

THE CARE OF CANS, PAILS, STRAINERS, ETC. Nothing is more diffi-cult to clean properly than cans, pails, strainers, etc., with the facilities at hand on the average farm. No matter how hard the good housewife may scrub the can, she will rarely succeed in cleansing it so thoroughly that it will be free from bacterial life. A solution of soda is commonly used, and it is effective in removing grease and other forms of dirt; but it has very little germicidal value. Even a four per cent solution is of little use as a germicide. The final scalding with hot water (one cannot say boiling water) is insufficient to kill the bacteria on the inner surface of the can and in the cracks and crevices which are usually present (see page 6). Steam, the best means of sterilizing cans, is not available on the farm. Hence the proper place for cleaning cans is at the factory, where all appliances are at hand for doing the work thoroughly and ex-peditiously. So far as we can see, this is the first step, and a very im-portant one, towards the removal of the trouble from the bitter Torula and other injurious organisms. Let all cans be thoroughly washed and *sterilized* before they leave the factory; and then let the factory insist, and see to it, that those in charge of the work on the farms supplying milk, (1) wash and scrub thoroughly (and occasionally scour) all pails, strainers, dippers, and other utensils used in handling the milk; (2) rinse all pails, strainers, dippers, etc., thoroughly with *boiling* water immediately before using; and (3) rinse the cans out thoroughly with *boiling* water a few minutes before putting the milk into them.

The practice of carrying ordinary, unpasteurized whey in milk cans from the factory to the farm cannot be too strongly condemned. So long as it is done, so long will there be trouble and serious loss from undesirable taints and flavors in milk; and the surprising thing is that good factories tolerate the practice. Let the whey be taken back in a can kept for the purpose; or, if that cannot be done, let all the whey be pasteurized at the factory by using the exhaust steam from the engine.

Utensils should be cleaned at the farm in the following manner: First, wash with water; then scrub well with a *hot* soda solution, using about two per cent. of soda (powdered concentrated ammonia may be used instead of soda); next empty out the soda washings, and scald with



A single germ.

Progeny of a single germ in 12 hours in milk cooled with cold water.

Progeny of a single germ in 12 hours in milk allowed to cool naturally.

FIG. 6.—Showing the effect of cooling milk on the growth of bacteria. The beneficial results of early chilling are manifest. (After Russell.)

boiling water; lastly empty the water out and allow the can to drain dry. Provide, if possible, some kind of cheap rack, to set the cans on, at an angle of about 45 degrees. On such a rack, the cans will dry out nicely and will not be much exposed to infection from dust, falling leaves, etc.

Do not, on any account, leave the water in the can; and do not use cloths or brushes in the final rinsing with the boiling water.

MILKING. The cows, before being turned out in the spring should have their flanks, udders, and tails well clipped. If before milking, the udder of the cow is rubbed with a damp cloth, the germs which are present on the hairy coat of the animal are prevented from falling off into the milk pail, as bacteria cannot leave a moist surface. Milking should always be done with clean, dry hands. Immediately after milking, the milk should be strained through a brass wire sieve, with several thicknesses of cheese-cloth on it, or, better, a woollen cloth; but these cloths must be carefully cared for and rinsed in boiling water *every day*. If they are not so cared for, their use should be dispensed with, as neglected cloths are an undoubted source of infection.

AERATION AND COOLING. Aeration and cooling are both very desirable. I have already spoken of the effect of temperature on the growth of the bitter Torula; and the following figures show the rate of growth of a single germ:

Rate of Growth of a Single Germ.

Temperature.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.
54° F.	4	6	8	26	435
97° F.	23	60	215	1830	3800

A reference to Fig. 6 will show the beneficial effect of early cooling; and I am strongly of the opinion that, if the milk is cooled sufficiently, the trouble from the bitter Torula will be largely overcome.

The Copenhagen Milk Company require that the milk be cooled to 40° F at the farm, and that when delivered at the city establishment, it shall not be more than 50° F. These figures are possibly too low for ordinary factory practice; but, if the milk were cooled even to 60° F. at the farm, or to a temperature, say, two degrees above that of the water supply, it would be a great benefit.

Most of the best coolers aerate as well as cool, and those properly constructed should cool the milk to a temperature of two degrees above that of the water used for cooling. Should the farmers be unwilling to incur the expense of buying coolers, an arrangement might be made, as at Copenhagen, Denmark, by which the factory would let coolers out on hire. These coolers, made of copper tinned over, are practically indestructible. With ordinary care, they last for ten or more years, and when the tin wears off they can be replated.

Most farmers now have windmills for pumping water, and a connection could be made with a barrel or tank suitably placed; and necessary, ice could be used in the barrel.

PLACE FOR MILKING. Throughout this bulletin, I have emphasized the fact that most patrons keep their milk cans, both before and after filling, in very unsuitable places, where there is constant danger of infection from harmful bacteria or from the absorption of objectionable odors.

The factory management should clearly and strongly forbid the following practices:

(1) The keeping of uncovered cans under trees.

(2) The keeping of uncovered cans near stables, piggeries, or barnyards.

(3) The keeping of cans for more than short periods on milkstands by the roadside.

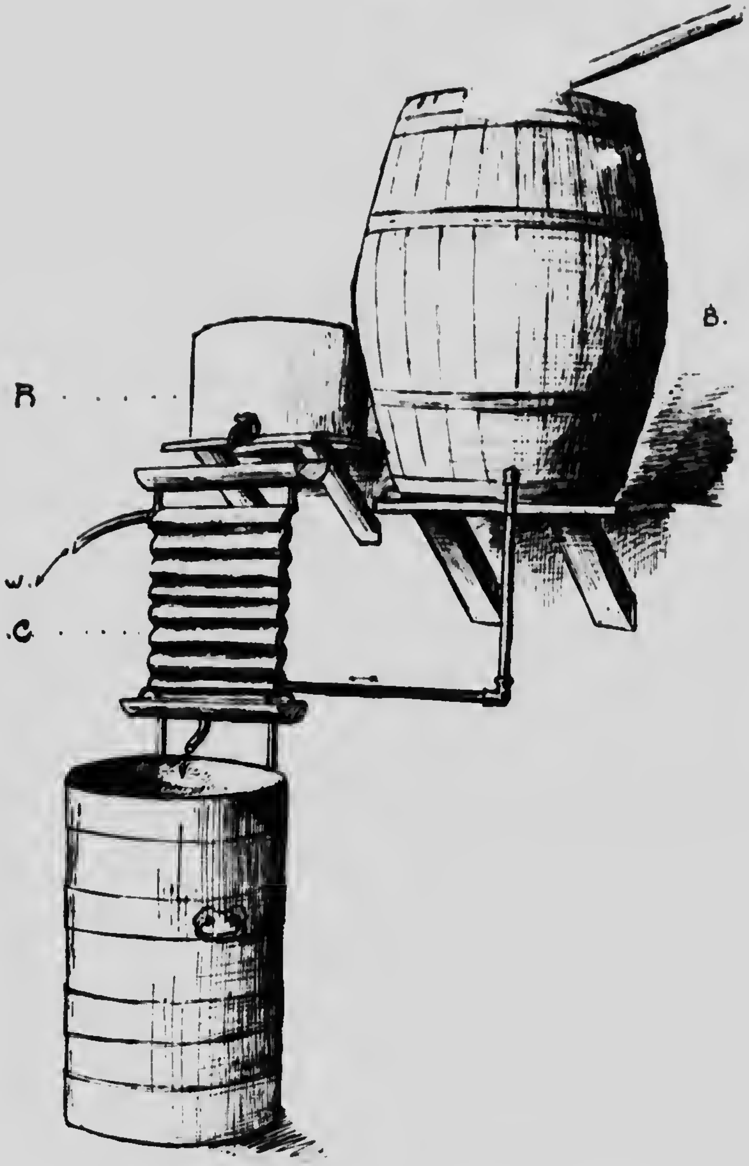


FIG. 7.—COOLING AND AERATING APPARATUS.

B. Barrel filled with cold water from well.
R. Reservoir to hold contents of milk pails.
C. Cooler and aerator. The milk passes over the corrugations.
W. Waste water pipe from the cooler. The water enters below on the right side and passes out at *W*.

Every farmer engaged in milk production should have a room in which to cool and aerate the milk immediately after milking, and to keep the filled cans until the time of putting them on the stand for the drawer to collect. This room need not be large or expensive, and it should be convenient to the stable or place of milking; but in no case should it be placed where there is a liability of infection from barnyard dust, or where the milk is in danger of absorbing stable, barnyard, or piggery odors. It should be sheltered from the prevailing wind A lean-to against a building often serves the purpose very well.

In this room, there should be space for the milk cooler and accessory apparatus, a spring weigh scale, and a requisite number of milk cans, a strainer, pails, etc.; and the milk, taken there immediately after milking, should be strained, and at once emptied into the tank above the cooler. In this way, the cooling and aeration would be finished a few minutes after the last pail of milk was drawn. Fig. 7 conveys some idea of what the interior of such a room should be like.

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