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# The Deleterious Effect of Freezing on Several of the Physical Properties of Milk

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# The Deleterious Effect of Freezing on Several of the Physical Properties of Milk

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ABSTRACT.—The freezing of milk containing flavors other than the desirable milk flavor causes such flavors to become more pronounced. The cream column of frozen milk decreases in length in direct relation to the freezing temperature and the length of time frozen. The freezing of the milk fat results in clumping of the globules. Many globules, when photographed, appear distorted in shape and irregular in their circumferences.

The object of this study was to determine the effect of freezing on the flavor and several of the physical properties of milk. The intensification of many of the undesirable flavors and odors which are most prevalent in winter was thought to be caused by the freezing of the milk.

Milk is frequently frozen while on the delivery wagon or subsequent to delivery. The length of time the milk is frozen depends upon the hour of delivery and the time when the milk is received by the consumer. This time commonly varies from one to five hours. The intensity of the freezing is made greater or is lessened depending upon the severity of the weather.

Casein in milk is a nucleo-protein and contains phosphorus. It is insoluble in water when free and uncombined. In milk it is combined with calcium in form of dicalcium caseinate, and is in suspension. Casein is responsible for the white color of milk and in part for its opacity. When milk is exposed to a low temperature, the calcium caseinate forms flakes, which, when the temperature is sufficiently low, are visible to the eye.

Fat is present in milk in an extremely finely divided condition, i. e., in an emulsion. The evidence is strong that the milk fat is derived from the phosphatid of the blood plasma.

In freezing, milk undergoes profound physical changes which become more pronounced with the length of time frozen, and it may be partially churned, that is, the complete emulsion which previously existed may be partially destroyed. The natural emulsion of the milk fat is never completely restored after thawing and the casein then appears in form of white flakes rather than in the original colloidal condition. The fat content of the upper layers may be three times as high as originally and much higher in the center portion of the milk than at the periphery. The emulsion of fat is destroyed much more rapidly when frozen than is the colloidal condition of the casein. Milk which has been frozen will decompose much more rapidly than unfrozen milk because of the injurious physical changes resulting.

The upper layer of milk after freezing has a lower specific gravity than in the original milk due to the increased fat content, although the total solids may not have changed materially.

The changes which take place in milk when frozen are permanent and the milk when thawed is never exactly the same as the unfrozen milk. The freezing point of milk is below that of water. The lower freezing temperature of milk is due to the presence of solids in suspension and solution.

When milk freezes, the freezing process is not complete as the external evidence may indicate. The water of the milk freezes first and the solids form a highly concentrated solution with a depression of the freezing point. The water freezes first at the outside on the wall of the vessel containing the milk; the solids, i. e., the fat, casein, albumin, milk sugar, etc. are forced toward the center giving a more concentrated solution, and this freezes at a lower temperature. As the freezing of the milk continues, the milk fat rises and is forced to the center. The central part of the frozen milk has a much higher specific gravity than the original milk, because of the higher solids, and somewhat less fat.

Milk has a lower surface tension than water largely on account of the proteins. That the individual fat globules are surrounded by a very thin film of protein, has been proven analytically by the higher nitrogen content of cream and butter as compared with milk. The protein films of the fat globules are not solid, but viscous and they cause a natural gathering of the fat in raw milk. These accumulations bring about the rapid gravitation of the fat and give the definite cream line.

Normal milk has a slight odor resembling the exhalations from the cow's skin and a slightly sweetish taste. During the colostral period and near the end of lactation, cow's milk may have a salty, bitter, or somewhat rancid, animal like taste. Milk of the individual cow may also be salty or bitter in advanced lactation, after abortion, in mastitis, and when the animal's digestion is disturbed. Milk with certain degrees of acidity will acquire a bitter, astringent taste in rusted vessels. A fishy taste is sometimes present when milk vessels are rusty or are not rinsed free of soap powder. Many aromatic feeds impart a characteristic odor and taste to the milk, for example, ensilage, rape, cabbage, beets, turnips, rutabagas, and carrots. Many undesirable odors are readily absorbed by the milk especially at a medium high temperature.

Many abnormal odors and tastes result from the growth of bacteria in milk. Activity of peptonizing bacteria may produce a bitter taste due to production of peptone, and later a foul and unpleasant odor and taste, the result of decomposition processes. Bacilli of the coli-aerogenous group produce an unclean, even nauseating, stable or manure-like odor and taste. Several organisms have been identified which produce bitter, soapy, oily, and burnt tastes and a stable-like odor and taste.

The literature furnishes few researches treating on this problem.

DuClaux[1]* reports frozen milk has a changed taste, but does not attempt to describe it, and says that there is not less cream in frozen milk. Farrington[2] found that freezing milk caused it to separate into two distinct parts, i. e., first, milk crystals largely compressed of water, with small admixtures of fat and serum solids, and second, liquid proteins containing nearly all of the milk proper. Heineiman[3] states milk undergoes chemical and physical changes which are more pronounced the longer it remains frozen, and that thawed milk never returns to its original state.

## EXPERIMENTAL WORK

**Effect of Freezing on Flavors in Milk.**—The flavors studied are those which are found more or less frequently in market milk. They are wash water, fly repellent, ragweed, silage, alfalfa, molasses, onions, animal heat, woody, oily, gasoline, metallic, and feces. These flavors were prepared in the following way. The wash water was obtained direct from a vat in which milk bottles had been previously washed; the fly repellent used was a commercial product; ragweed, ensilage, alfalfa, and onion flavors were obtained from the plants; and the woody flavor from pine shavings. The metallic solution was obtained by immersing metal shavings. A solution from each was sterilized to destroy all life and to prevent developments in the milk of an off flavor from some other source. Ordinary oil and gasoline were used as gasoline is sometimes transported in milk cans and the oil may come from the separator, or other sources. The animal heat and feces flavors were readily obtained by exposing the milk in open pails for a brief time in the barn.

The procedure followed in determining the relation of freezing to the intensification of these off flavors was as follows: fresh whole milk was received and judged to observe that it was free from undesirable flavors. This milk was then standardized to test 3.70 per cent butterfat, to give assurance that no differences in our observations could be attributed to the differences in the butterfat content. A skimmilk free from undesirable flavors was used in standardizing the whole milk. The milk when found satisfactory, was divided and equal amounts placed in sterilized glass vessels, each having a known capacity. The glass containers were then marked as follows: check samples of original milk not to be frozen; check samples containing known flavor not to be frozen; samples containing

*List of references will be found on page 14.

flavor to be frozen and thawed at 45° F. (7.22°C.), 60° F. (15.55° C.). 75° F. (23. 89°C.), 90°F. (32.22°C.). All determinations were made in duplicate. The containers were stoppered to prevent the volatilization of any flavors. The various temperatures were used to determine if they might increase or lessen the intensification of the off flavors due to the variance in the time that might be required to thaw the respective samples.

The amount of each flavor under study added to the measured samples of milk, with the exception of the checks, was just sufficient to give the milk a flavor perceptible to the taste. The samples were then frozen at a constant temperature of 10°F. (-12.22°C.) for a period of three hours, then they were thawed at the respective temperatures noted.

**Effect of Freezing on the Cream Line of Milk.**—To determine the effect of freezing on the cream line fresh samples of milk, testing 4.50 per cent butterfat, were obtained and cooled to 42°F. (5.55°C.) Ten calibrated 100-ml graduates were filled with milk to the 100 ml mark and allowed to remain quiet for twelve hours at 42°F. (5.55°C.) to permit gravitation of the milk fat. This length of time was required to assure the maximum creaming of the milk. Eight of the graduates containing the milk were then frozen at a constant temperature and for the desired lenght of time. Two graduates were retained at the cooling room temperature as controls. When the freezing process was complete the graduates were removed and held at a temperature of 60°F. (15.55°C.). Observations were made and the graduates photographed. Observations were again made after one, three, and twenty-four hours respectively. Milk was frozen in the graduates for three and five hours at 0°F. (-17.78°C.), 6°F.(-14.44°C.), 10°F. (-12.22°C.) 15°F. (-9.44°C.), 20°F.(-6.67°C.), 25°F. (-3.89°C), 30°F. (-1.11°C.). These periods of freezing and temperatures were decided upon as representative of common conditions.

Table one shows the effect of freezing on the cream line at different temperatures when frozen for three and five hours respectively.

TABLE 1.—EFFECT OF FREEZING MILK AT VARIED TEMPERATURES FOR DIFFERENT PERIODS OF TIME

| Temperature Frozen in Degrees | Frozen 3 hours Percentage loss after thawing | | | Frozen 5 hours Percentage loss after thawing | | |
|---|---|---|---|---|---|---|
| | 1 hr. | 3 hr. | 24 hr. | 1 hr. | 3 hr. | 24 hr. |
| 0°F. (-17.78°C) | 39.00 | 42.44 | 45.90 | 40.75 | 42.65 | 45.90 |
| 6°F. (-14.44°C) | 38.84 | 41.82 | 43.16 | 40.12 | 42.47 | 45.40 |
| 10°F. (-12.22°C) | 31.68 | 33.33 | 38.34 | 39.83 | 41.24 | 43.40 |
| 15°F. (-9.44°C) | 24.90 | 29.70 | 33.20 | 28.70 | 33.80 | 38.88 |
| 20°F. (-6.67°C) | 15.78 | 24.65 | 28.67 | 18.00 | 26.19 | 32.70 |
| 25°F. (-3.89°C) | 8.31 | 13.98 | 25.57 | 15.90 | 27.90 | 30.10 |
| 30°F. (-1.11°C) | 0.58 | 4.25 | 12.19 | 2.21 | 5.89 | 13.04 |

## DISCUSSION OF RESULTS

When the milk samples to which no flavors had been added and those containing the different flavors were judged the intensity of the aroma and flavor and the condition of the body of the cream column were observed. The data indicated that in every instance the samples of the original milk which were not frozen had a clean, desirable aroma and flavor. The check samples of the original milk which had been frozen were free from an unclean aroma and flavor, but possessed a flavor which was flat, due probably to the watery condition of the samples, caused by the freezing and a separation of the liquid and solids of the milk. Samples containing the different flavors, but which had not been frozen did not show any perceptible change due to aging. The samples to which had been added the various flavors and which had been frozen had very pronounced and greatly intensified aromas and flavors, common to, or typical of the particular flavor added. This was true of every sample frozen and then thawed at 45°F. (7.22°C.), 60°F. (15.55°C.), 75°F. (23.89°C.), 90°F. (32.22°C.). The intensity of the aroma and flavor imparted by each sample diminished with a lowering in the thawing temperatures, the most pronounced aroma and flavor being prevalent in the samples thawed at 90°F. (32.22°C.)and the least pronounced in the samples thawed at 45°F. (7.22°C.).

The samples to which the wash water flavor had been added gave an aroma and flavor which was tallowy and characteristic of wash water; the samples containing fly repellent caused a biting sensation on the tongue; ragweed gave a flavor that was sickening and nauseating; as did the samples prepared from animal heat and feces. A resin flavor was obtained when a woody solution had been added to the milk. The flavor of gasoline, metallic filings, and oil produced flavors which were pronounced very undesirable and offensive.

The intensification of the undesirable aroma and flavors in the frozen milk may be explained by the probable volatilization of some slightly volatile fats or substances in the milk which has served as a buffer in causing the objectionable flavors not to become apparent when the milk was in an unfrozen state. This explanation is feasible in that milk having a very desirable flavor before freezing has a flat taste after having been frozen. The change in the flavor may also have been due, in part at last, to a precipitation of a part of the proteins and mineral constitutents. The question of the solidity of the milk fat concerns itself in these deductions, inasmuch as a fat in the milk at ordinary cooling room temperature is only partially solidified. The partially solidified fat globules permit a partial volatilization, this becoming more apparent as the temperature is raised. This explains why milk when thawed at higher temperatures imparts the several flavors with greater intensity than did the same samples when thawed at lower temperatures. The data appears to indicate conclusively that freezing of milk causes a decided intensification of any flavor which is present in milk at the time of freezing.

When the temperature is sufficiently low to cause freezing, a separation of the solids results, the fat is forced to the center and protrudes above the neck of the bottle as the freezing continues. The pressure applied as a result of the freezing process partially destroys the emulsion of the milk fat. When the milk is exposed to a temperature above freezing, thawing occurs, and the ingredients of the milk endeavor to resume their original state. Observations made of the milk after thawing indicate that a physical change has taken place. The serum has changed from its original color to that of a bluish and watery appearance. The detrimental effect on the cream line is even more serious and is very apparent. When milk was frozen for a period of three hours at 0°F. (-17.78°C.) and then allowed to thaw at 60°F. (15.55°C.) for one hour, the decrease in the lenght of the cream line, in comparison with the original, was 39 per cent; after standing at 60°F. (15.55°C.) for three hours the decrease was 42.44 per cent, and after setting twenty-four hours the loss was 45.90 per cent. The same milk frozen at 0°F. (-17.78°C.) for five hours and thawed at 60°F. (15.55°C.) for one hour resulting in a diminishing of the cream line of 40.75 per cent; after three hours 42.65 per cent and after twenty-four hours 45.90 per cent.

The data in Table 1 show that as the freezing temperatures were varied from 0°F. (-17.78°C.), 6°F.(-14.44°C.), 10°F. (-12.22°C.), 15°F. (-9.44°C.) 20°F. (-6.67°C.), 25°F. (-3.89°C), 30°F. (-1.11°C.) the decrease in the length of the cream line in graduates thawed at 60°F. (15.55°C.) and observations made after one, three and twenty-four hours diminished with definite regularity with respect to the freezing temperature and the time elapsing between observations. The same was true in the samples frozen for five hours at the same temperatures. The body of the cream of every sample frozen and thawed, when observed after one hour was granular, porous, and decidedly coarse. The upper and lower surface of the cream columns were irregular and ragged. As time elapsed these undesirable characteristics gradually disappeared. When the last observations were made after twenty hours the body in almost every sample was described as being quite smooth, free from the marked granular appearance, and the surfaces of the cream column regular and level except in the milk frozen at the extremely low temperatures, in which instances the effect of the freezing remained slightly apparent.

The decrease in the length of the cream column may be explained in several ways. When the milk is subjected to freezing temperatures, separation of solids takes place, the fat finding its way to the outer and upper strata of the frozen column. The emulsion of the fat is partially or totally destroyed. Micro-photographs show that the fat has been disturbed and distorted and as a result of the partial destruction of the emulsion grouping of the fat globules has taken place. The grouping of the fat globules may also be made possible by a partial preciptation of the free casein or the precipitaton of the albumen which is then partially or almost entirely removed from the column of milk fat.

As a result of the removal of a part of the casein the resistance which had been previously offered by the presence of the casein had been removed and the butterfat globules were permitted to come closer together causing them to occupy less space and to rise more readily, giving a decrease in the length of the cream column, with a lowering of the freezing temperature and increase in the length of freezing time. It seems reasonable to believe that the precipitation of the casein may be expected to be even greater, the groups or clumps of butterfat larger and more numerous. The clumps or groups of butterfat globules may be dispersed in the serum below the fat column or even embedded in the precipitated casein which no longer forms a part of the remaining cream column.

Figure 1 shows graduated milk samples before freezing and after allowing the milk fat to gravitate for twelve hours. The regularity of the depth of the columns of cream and the evenness of the lower level of the columns are well defined.

The freezing of the cream column, as shown in Figure 2, causes the column of fat to be extended, the upper and lower levels to become very uneven and the butterfat to appear granular and porous. A butterfat test of the upper strata would greatly exceed that of any other portion of the column as the freezing forces most of the fat to that area.

The diminishing of the cream column is very apparent in Fig. 3, contrasted with the unfrozen samples, the decided irregularity of the upper and lower levels and the openness of the column proper, accompanies a diminishing of the length of the column. The body of the cream even at this period continues to be somewhat granular and coarse.

Figures 4 and 5 show with definite regularity that the cream columns of milk which have been frozen upon standing for different periods of time resume evenness at the upper and the lower surfaces. The granular and porous condition of the body has become less apparent and the column of cream decreases to almost one half its original length.

Figure 6 is a microphotograph of whole milk before freezing. The individual globules are uniform in size and have even circumferences.

In Fig. 7 the effect of the freezing is very evident. The fat globules are no longer uniform in size. They are greatly distorted, irregular in shape and have uneven circumferences. Grouping and clumping of the globules as a result of pressure applied during the freezing process is very apparent.

Figure 8 shows that in prolonging the time of freezing, the fat globules appear in larger groups and clumps, are greatly distorted and more irregular in shape and size. As freezing progresses a separation of solids from the liquid phase occurs and exerts sufficient pressure to bring about these marked physical disarrangements.

## CONCLUSIONS

The freezing of milk with even a mild flavor intensifies that flavor.

The freezing of the milk produced flavors which were objectionable and oftentimes very offensive.

The intensification of undesirable flavors as a result of freezing is a menace to the market milk industry.

The cream column of milk is diminished as a result of freezing.

The decrease in the length of the cream column becomes greater as the freezing temperature is lowered and the time of freezing prolonged.

The body of the cream column during the first several hours after thawing is unsatisfactory, because of its granular and porous appearance.

Freezing causes the fat globules to form clumps or groups and become distorted and irregular in shape and size.

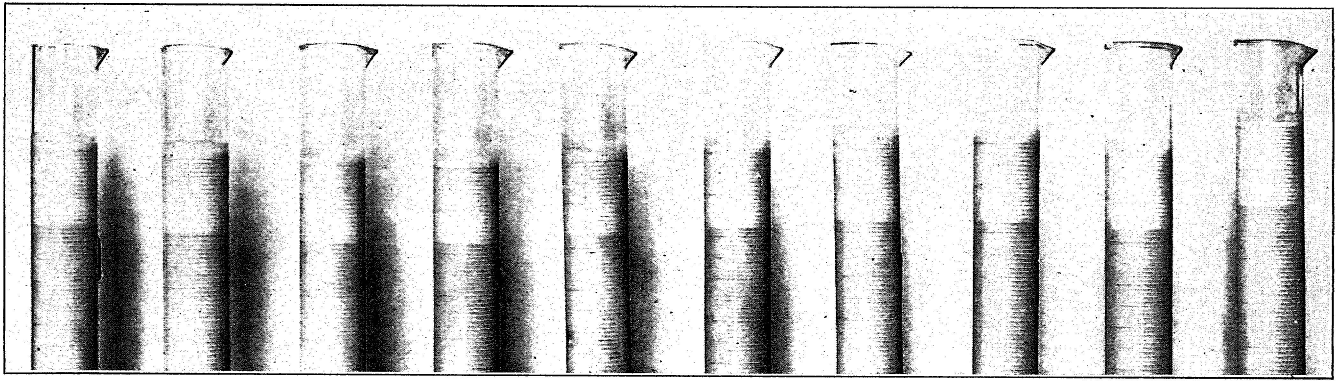

Fig. 1.—Whole Milk, butterfat test 3.80 per cent, held 42°F. (5.55°C.) for twelve hours.

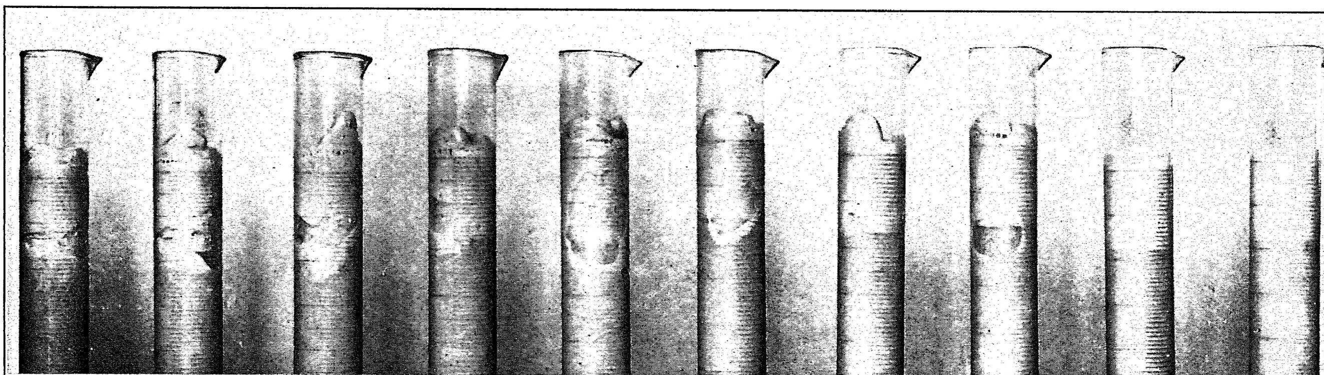

Fig. 2.—Whole milk, butterfat test 3.80 per cent, held at 42°F. (5.55°C.) tor twelve hours and then frozen for five hours at 0°F. (-17.78°C.) The two samples on right are originals.

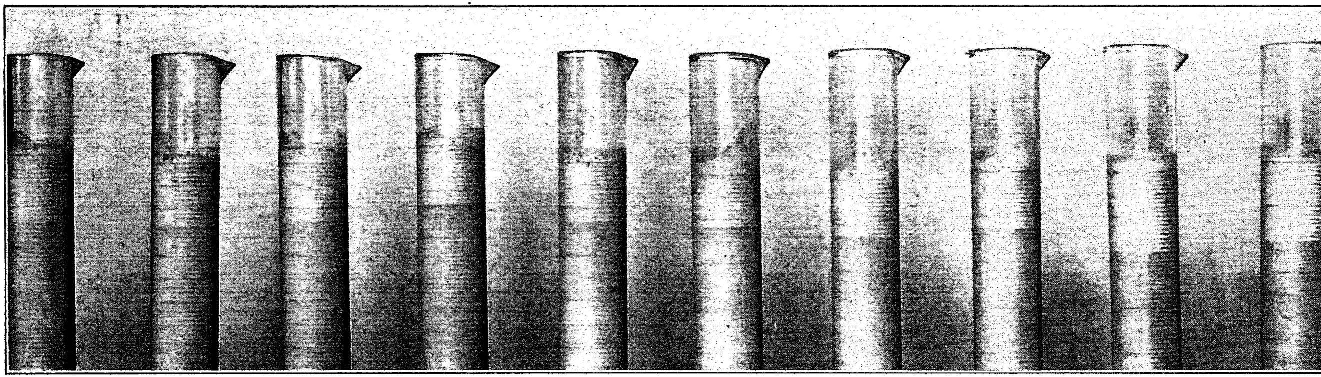

Fig. 3.—Whole milk, butterfat test 3.80 per cent, held at 42°F. (5.55°C.) for twelve hours, frozen for five hours at 0°F. (-17.78°C.) and then exposed to a temperature of 60°F. (15.55°C.) for one hour.

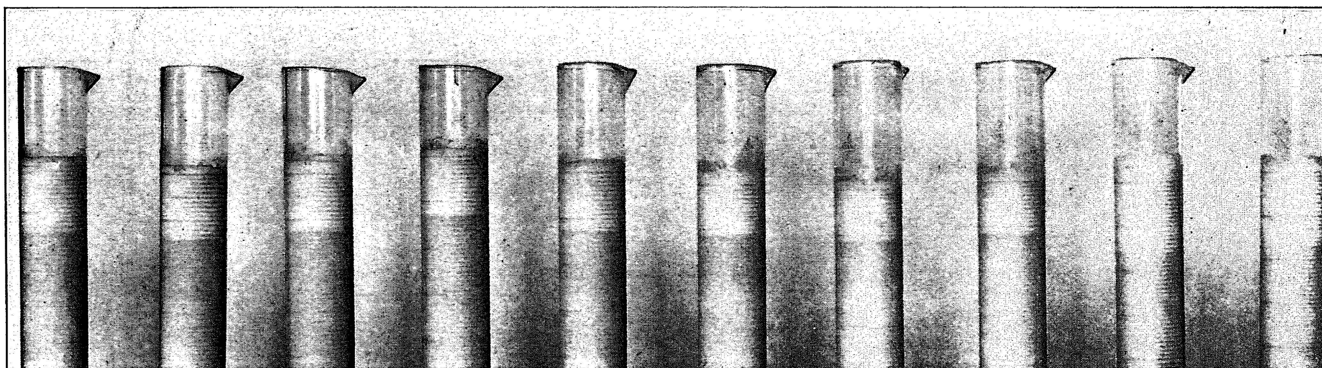

Fig. 4.—Whole milk, butterfat test 3.80 per cent, held at 42°F. (5.55°C.) for twelve hours, frozen for five hours at 0°F. (-17.78°C.) and then held for three hours at 60°F. (15.55°C.)

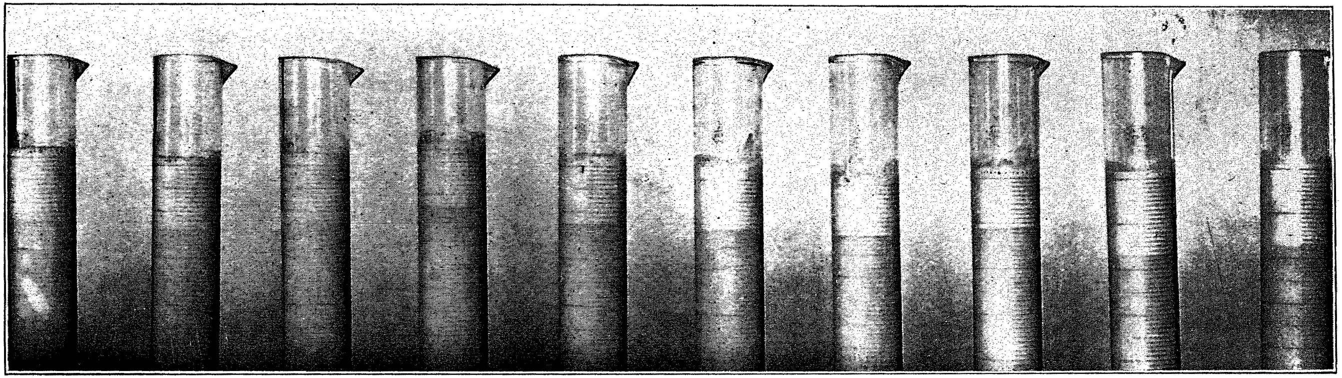

Fig. 5.—Whole milk, butterfat test 3.80 per cent, held at 42°F. (5.55°C.) for twelve hours frozen for five hours at 0°F. (-17.78°C.) and then held for twenty-four hours at 60°F. (15.55°C.)

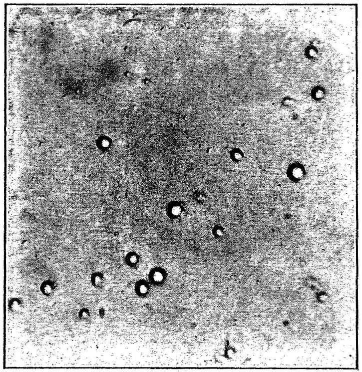

Fig. 6.—Butterfat globules of whole milk, butterfat test 3.80 per cent before freezing.

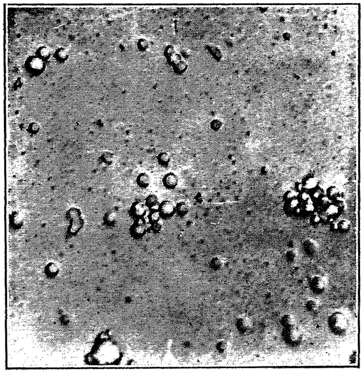

Fig. 7.—Butterfat globules of the whole milk frozen three hours at 0°F. (-17.77°C.)

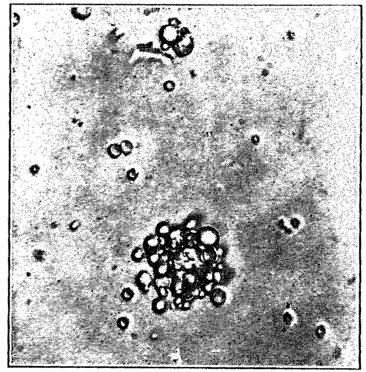

Fig. 8.—Butterfat globules of the whole milk frozen five hours at 0°F. (-17.78°C.)

## BIBLIOGRAPHY

1. DuClaux, E., *Sur le Lait Congeli*; Annales del'Institute Pasteur, 10, 1896, p. 393.
2. Farrington and Woll, *Testing Milk and Its Products*, 1918, p. 27.
3. Heineiman, P. G.; Milk 1919, p. 128.