# JOURNAL OF
# DAIRY SCIENCE





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# LIPOLYTIC ACTIVITY IN MILK AND CREAM

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Lipase in milk was first convincingly demonstrated in work reported by Maass (1) in 1909. Sterilized cream, with formalin as a preservative, on inoculation with raw cream, showed increases in acidity on incubation, with the increases proportional to the inoculum. Greater increases were caused by cream from milk late in the lactation period. However, as late as 1922 Palmer (2) failed to demonstrate the presence of lipase in milk, but Rice and Markley (3), reporting in the same journal issue, demonstrated lipase by the increase in acidity that developed in a substrate of boiled cream, saturated with sugar, and inoculated with raw milk. Later in the same year Palmer (4) reported lipase in milk taken near the end of the lactation period, and attributed the development of a bitter flavor and rancid odor in such milk mainly to butyric acid set free by lipolysis. Since then the presence of lipase in milk has been generally recognized, and many interesting facts have been reported concerning its activity.

In studying the lipolytic activity of milk various procedures have been used. Early methods have been reviewed by Rice and Markley. In the early work various preservatives, including chloroform and formaldehyde, were used to exclude bacterial action, but chloroform was found to retard lipase action (5), and formaldehyde also limits it. The finding that formaldehyde limits lipolysis to various extents in different milk samples led Herrington and Krukovsky (6) to postulate at least two lipases in milk. In more recent work chemical preservatives have been avoided by using sugar-saturated-cream as the substrate (3), or by making the observations on the milk or cream itself, allowing lipolysis to proceed at low temperatures to limit bacterial activity (6), or by using a buffer substrate containing tributyrin (7). The extent of lipolysis has usually been measured by titration with standard alkali, the increase in titer being attributed to free fatty acids. The titrations have been applied to the sample directly, with or without the addition of organic fat solvents to make the free fatty acids more accessible, or to the fat obtained from the sample by churning and oiling-off the resulting butter (6), or to the steam distillate from the sample (7). The decrease

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in surface tension resulting from lipolysis in milk has been suggested as a measure (8).

The view has persisted that lipolytic activity in milk is greatest near the end of the lactation period. Since the work of Maass (1) and Palmer (4) various reports have tended to confirm this view. Sharp and DeTomasi (9) in reporting on lipolysis in raw cream adopted this view. Hileman and Courtney (10) attributed the maximum lipolytic activity in December and January in part to the lactation cycle. Krukovsky and Sharp (11) attributed the slow churning of cream from cows in advanced lactation to lipolytic action. Yet studies of this factor have failed to establish a close relationship between lipase content and the lactation period. Mattick and Kay (7), working with a buffered tributyrin substrate, failed to find a higher lipase (butyrinase) content in advanced lactation. Pfeffer, Jackson and Weckel (12), working with sugar-saturated-cream substrate, failed to find a relationship between lipase content and the stage of lactation. Even when the fat in the milk sample itself served as the substrate, thereby involving differences in fat content, fat globule sizes and surface area of the fat, Herrington and Krukovsky (6) found no relationship between lipolysis and the stage of lactation, stage of gestation, or the amount of milk produced by each cow.

The study of the lipase content of milk is complicated by the factors that greatly alter the activity of the lipase that is present. Dorner and Widmer (13) found that homogenization of raw milk greatly increased subsequent lipolysis. This has been confirmed repeatedly and is generally assumed to be due to the increased area of contact between the fat and the aqueous phase containing the lipase, but may possibly involve other factors (12). Krukovsky and Sharp (14) have reviewed the literature pertaining to activation of lipase by shaking and have confirmed and extended it to show that shaking is particularly effective when the fat is in the liquid state. Herrington and Krukovsky (6) found less lipolysis in milk that was cooled rapidly by means of a tubular cooler as compared with the same milk cooled in air at 0° C. or in water at 0° C. They also found that precooling followed by warming activated the lipase as subsequently observed at lower temperatures. The effectiveness of the precooling increased as the temperature was lowered from 25° C. to 0° C., but the duration of the precooled condition seemed to be of little importance. The effectiveness of the warming after the precooling increased up to 30° C. and beyond this the effect decreased so that at about 37° C. the effect of the precooling was undone. The earlier finding by Sharp and DeTomasi (9) that less lipolysis takes place in cream from milk forewarmed to 110°–115° F. and separated at 75°–85° F. than in cream from the same milk forewarmed and separated at 75°–85° F., might be due to these activation factors. Davies (14) found that lipolysis was inhibited by heavy metals, especially copper. Herrington and Krukosvky (6) found that 0.2 and 0.4 p.p.m. of copper reduced lipolysis at 0° C. about 20 per cent.

## METHOD

In this study lipolysis was observed in (a) samples of milk and cream held in a refrigerator (near 0° C.), and (b) sugar-saturated-cream inoculated with samples of skim milk, milk or cream, and held at 37° C. The sugar-saturated-cream was prepared from 40 per cent cream by adding 2 parts of sucrose to every 1 part of water naturally present in the cream, heating to 145° F., homogenizing at 1,500 lb. pressure, portioning the product into bottles, and sterilizing in streaming steam for 30 minutes. In this manner the substrate could be prepared and kept in batches of sufficient size to serve several series of experiments.

Lipolysis was followed by determining the titer of the steam distillate at the start and after holding. For this purpose 20 grams of the sample were acidified with 1.2 cc. N/1 sulphuric acid and then subjected to steam distillation, collecting 200 cc. of distillate in a closed receiving vessel fitted with a soda lime tube. This distillate was titrated with N/50 sodium hydroxide from a microburette using 5 drops of 1 per cent phenolphthalein as indicator. The following precautions were found essential: Use glass equipment fitted throughout with glass joints. Generate the steam from distilled water, and before placing the 20 grams of the sample in the distilling flask, free the water and the equipment from carbon dioxide by distilling over at least 150 cc. of distillate. Keep the distilling rate reasonably uniform, and keep the volume of the sample substantially constant by placing a small flame under the distilling flask. With these precautions duplicate determinations consistently checked within 0.03 cc.

While this procedure measures only the volatile, soluble acids, the results are comparable in a series of samples where the fat is the same. In the case of the sugar-saturated-cream experiments the fats differed only to the slight extent that fat was introduced in the inoculum.

In experiments reported in tables 2 and 5, where cream samples were held at refrigerator temperature, fat samples were obtained by churning and oiling-off for a comparison of the acid number of the fat by the Herrington-Krukovsky procedure with the titer of the steam distillate from the cream samples. The results were quite closely parallel.

## EXPERIMENTAL

### *The Effect of Tributyrin and Formaldehyde on Lipolysis*

As a check on the method of procedure here adopted and on the results reported in the literature, the sugar-saturated-cream substrate was inoculated with 40 per cent raw cream, without and with further additions of tributyrin and formaldehyde as shown in table 1.

Formaldehyde limited lipolysis to about a third or a half, and tributryin greatly increased lipolysis as measured by the titer of the steam distillate. In both cases no distinction was apparent between the two levels of addition.

TABLE 1

*The effect of tributyrin and formaldehyde on lipolysis*

| Sample 100 grams substrate inoculated with 10 grams 40% raw cream plus | Acidity of steam distillate, cc. N/50 NaOH, after incubation at 37° C. for | | | |
|---|---|---|---|---|
| | 0 hr. | 24 hr. | 48 hr. | 96 hr. |
| Control, no further additions | 0.50 | 2.76 | 4.00 | 5.90 |
| Formalin,* 0.05 cc. | 0.49 | 1.35 | 1.47 | 1.79 |
| Formalin, 0.15 cc. | 0.50 | 1.37 | 1.49 | 1.77 |
| Tributyrin, 1 gram | 1.51 | 10.27 | 16.33 | 18.69 |
| Tributyrin, 2 grams | 2.23 | 10.27 | 16.33 | 18.69 |
| Control, no further additions | 0.38 | 2.31 | 3.87 | 4.99 |
| Formalin, 0.15 cc. | 0.38 | 1.16 | 1.45 | 1.80 |
| Tributyrin, 1 gram | 1.69 | 9.43 | 15.21 | 18.00 |
| Control, no further additions | 0.46 | 5.41 | 6.63 | 7.01 |
| Formalin, 0.15 cc. | 0.46 | 1.86 | 2.61 | 2.93 |
| Tributyrin, 1 gram | 1.96 | 12.60 | 17.17 | 21.03 |

* Containing 37 per cent formaldehyde.

### *A Comparison of Machine Separated and Gravity Separated Cream*

Since the lipase is present in the aqueous plasma of cream but may be associated with the fat to a greater or lesser extent by adsorption, it was of interest to compare lipolysis in separator cream and gravity cream from the same original milk. The extreme difference in the forces applied in the two methods of separating the cream from the milk might affect the distribution of the lipase.

The milk and cream used in this experiment represents mixed milk from a number of dairies as received for the commercial operations of the University Creamery. With the milk forewarmed to 30–35° C. in the commercial separating operations, cream and skim milk were obtained simultaneously and at the same moment some of the same milk, but not forewarmed, was taken for gravity creaming. These samples were obtained in the morning, were cooled to and held at 2–3° C. until mid-afternoon when the gravity cream was removed from the milk. The gravity cream was tested for fat (usually about 20 per cent fat) and the separator cream in each comparison was standardized to the same fat content by adding the proper amount of the separator skim milk. A portion of the standardized, separator cream was pasteurized at 145° F. for 30 minutes to serve as a control. The three samples,—pasteurized cream, separator cream and gravity cream, were held at 2–3° C. for lipolysis observations by two methods: (a) by determining the titer of the steam distillate from 20 grams of the cream, and (b) by churning the cream and determining the acid number of the fat by the method of Herrington and Krukovsky (6). Eight such comparisons were made, and in each case the gravity cream showed significantly greater lipolysis than the separator cream in spite of the fact that the separator cream was favored by

the activating effect of forewarming while the gravity cream was not. Typical results are given in table 2.

TABLE 2

*A comparison of machine separated and gravity separated creams*

| Sample | Acid number* of fat after holding at 2–3° C. for | | Acidity of distillate† after holding at 2–3° C. for | |
|---|---|---|---|---|
| | 0 hr. | 48 hr. | 0 hr. | 48 hr. |
| Control (pasteurized cream) | 0.38 | 0.40 | 0.69 | 0.71 |
| Separator cream | 0.38 | 0.60 | 0.69 | 8.63 |
| Gravity cream | 0.38 | 0.68 | 0.71 | 9.11 |
| Control (pasteurized cream) | 0.40 | 0.40 | 0.58 | 0.61 |
| Separator cream | 0.40 | 0.56 | 0.57 | 7.49 |
| Gravity cream | 0.40 | 0.61 | 0.60 | 9.36 |
| Control (pasteurized cream) | 0.36 | 0.36 | 0.71 | 0.71 |
| Separator cream | 0.36 | 0.60 | 0.71 | 8.37 |
| Gravity cream | 0.38 | 0.69 | 0.74 | 11.29 |
| Control (pasteurized cream) | 0.34 | 0.35 | 0.87 | 0.89 |
| Separator cream | 0.34 | 0.58 | 0.87 | 6.83 |
| Gravity cream | 0.33 | 0.63 | 0.91 | 9.12 |

* Cc. of N/20 NaOH to titrate the acidity in 5 grams of fat.
† Cc. of N/50 NaOH to titrate the steam distillate from 20 grams of cream.

### *The Effect of Shaking on Lipolysis*

The effect of shaking on lipolysis was observed on samples of milk, on samples of the same milk in sugar-saturated-cream, and on samples of cream and corresponding skim milk in sugar-saturated-cream. All of these samples, including those prepared with the sugar-saturated-cream substrate, were held at 3–4° C. except during the shaking period, when all of them including the controls (unshaken), were brought to 25° C. The shaken samples were shaken during the first three hours of each 24-hour period in a mechanical shaker in which they travelled horizontally in a path 1½ inches long at the rate of 195 strokes (complete cycles) per min. Lipolysis was measured by the steam distillation and titration procedure applied at 0, 24, 48, and 96 hours. The results are given in table 3.

Where the milk samples without additions were held, shaking increased lipolysis, but where the milk, cream or skim milk was used to inoculate the sugar-saturated-cream substrate, shaking caused a decrease. In the former case the increase was apparently due to washing the fat surface free from end products; increased fat surface due to dispersion of the fat could hardly be involved since the conditions were conducive to churning. In the inoculated substrate this apparently is not a limiting factor, and shaking then produces the detrimental effect that is quite common for enzyme action.

TABLE 3

*The effect of shaking on lipolysis*

| Sample | Acidity of steam distillate in cc. of N/50 NaOH after holding* time of | | | |
|---|---|---|---|---|
| | 0 hr. | 24 hr. | 48 hr. | 96 hr. |
| Whole milk No. 1 | 1.22–1.22† | 2.27–3.22 | 3.26–5.05 | 4.16–6.69 |
| Whole milk No. 2 | 1.72–1.72 | 1.91–2.72 | 2.22–3.07 | 2.42–3.57 |
| Whole milk No. 3 | 1.46–1.46 | 1.80–2.76 | 2.26–3.97 | 2.59–4.60 |
| Whole milk No. 4 | 1.33–1.33 | 2.35–2.41 | 3.88–4.08 | 4.26–5.69 |
| Whole milk No. 5 | 1.41–1.41 | 2.43–2.99 | 4.03–4.47 | 4.69–5.99 |
| Whole milk No. 6 | 1.09–1.08 | 2.17–2.81 | 3.64–4.00 | 4.19–5.71 |
| Whole milk No. 7 | 1.26–1.26 | 2.22–2.93 | 3.67–4.20 | 4.39–5.91 |
| 100 grams of sugar-saturated-cream plus 10 grams of: | | | | |
| Whole milk No. 4 | 1.89–1.89 | 2.43–2.36 | 3.91–3.42 | 5.64–4.63 |
| Whole milk No. 5 | 1.91–1.91 | 2.78–2.65 | 4.12–3.68 | 6.94–5.78 |
| Whole milk No. 6 | 1.80–1.80 | 2.31–2.19 | 3.45–2.97 | 6.44–5.23 |
| Whole milk No. 7 | 1.83–1.83 | 2.75–2.70 | 3.54–2.75 | 6.36–5.39 |
| Whole milk No. 8 | 1.84–1.84 | 2.44–2.29 | 3.30–2.98 | 5.63–4.77 |
| 110° F. cream from No. 8‡ | 2.04–2.04 | 2.76–2.59 | 3.34–3.04 | 4.78–4.77 |
| 110° F. skim milk from No. 8 | 1.93–1.93 | 2.59–2.40 | 3.11–2.97 | 5.18–4.56 |
| 75° F. cream from No. 8 | 1.80–1.80 | 2.51–2.41 | 3.44–3.27 | 5.22–4.85 |
| 75° F. skim milk from No. 8 | 1.84–1.84 | 2.63–2.30 | 3.38–2.85 | 5.62–4.91 |

* The holding temperature was 3–4° C. except during the first 3 hours of every 24-hour period when the temperature was 25° C.

† In these pairs of figures the first is the value found for the unshaken sample, the second for the corresponding shaken sample.

‡ The temperature here refers to the temperature of the milk at the time of separation.

### *The Effect of Temperature on Lipolysis*

To observe the effect of the several temperature levels used in this and other work, sugar-saturated-cream was inoculated with whole milk in the proportion of 10 to 1 and held at refrigerator (3–4° C.), room (27° C.), and incubator (37° C.) temperatures. At 0, 24, 48, and 96 hours, portions were steam distilled and the distillate titrated. Duplicate determinations in this case were made by using 2 samples at each temperature with the samples

TABLE 4

*The effect of temperature on lipolysis*

| Temperature °C. | Acidity of the steam distillate in cc. of N/50 NaOH after holding at the specified temperature for | | | |
|---|---|---|---|---|
| | 0 hr. | 24 hr. | 48 hr. | 96 hr. |
| 3–4 | 0.83–0.83* | 3.42–3.54 | 4.05– 4.22 | 4.26– 4.31 |
| 27 | 0.83–0.84 | 3.85–4.13 | 8.81– 9.28 | 9.78–10.10 |
| 37 | 0.83–0.83 | 4.04–4.66 | 10.67–11.01 | 12.08–12.31 |
| 3–4 | 0.69–0.69 | 2.78–2.79 | 3.47– 3.22 | 3.91– 3.91 |
| 27 | 0.69–0.69 | 3.11–3.01 | 8.01– 7.88 | 10.10– 9.89 |
| 37 | 0.68–0.69 | 3.64–3.56 | 9.16– 8.97 | 12.49–12.38 |

* Paired figures represent duplicate determinations but with different batches of substrate (batches 1 and 2 in the first series, batches 1 and 3 in the second).

prepared from different batches of substrate but inoculated with the same milk throughout. The experiment was repeated using one of the former batches of substrate and a new batch. The results are given in table 4.

The results were in harmony with expectations; lipolysis was most extensive at 37° C., slightly less at 27° C. and decidedly less at 3–4° C. in the case of 48 and 96 hour holding. However, the difference after only 24 hours holding was surprisingly small. A slight difference in the duplicate determinations is attributable to the substrates; in the first series batch 1 consistently yielded lower results than batch 2, and in the second series, batch 1 yielded higher results than batch 3 in practically all cases.

### *The Effect of pH on Lipolysis*

To observe the effect of hydrogen ion concentration on the rate of lipolysis a series of samples, ranging from about pH 6.2 to 9.0, were prepared from 40 per cent raw cream with appropriate additions of N/1 lactic acid or N/1 sodium hydroxide as required. The samples were held at 3–4° C. for 48 hours. At 0 and 48 hours the pH of the samples was determined at room temperature by means of the quinhydrone electrode, and 20 gram portions were steam distilled and the distillate titrated with N/50 NaOH. The remainder of the cream at 48 hours was churned and the fat used to determine its acid number by the Herrington and Krukovsky procedure. The results from a number of such series indicate that the optimum pH is at pH 8.4 to 8.6. Typical results are given in table 5.

TABLE 5

*The effect of pH on lipolysis*

| Sample | pH after holding at 3–4° C. for | | Acidity of distillate in cc. N/50 NaOH after | | Acid No. of the fat after |
|---|---|---|---|---|---|
| | 0 hr. | 48 hr. | 0 hr. | 48 hr. | 48 hr. |
| 1 | 6.19 | 6.19 | 0.46 | 0.69 | 0.60 |
| 2 | 6.25 | 6.23 | 0.46 | 0.81 | 0.75 |
| 3* | 6.61 | 6.58 | 0.46 | 2.14 | 1.20 |
| 4 | 6.81 | 6.62 | 0.46 | 2.84 | 1.85 |
| 5 | 7.01 | 6.69 | 0.46 | 3.11 | 1.98 |
| 6 | 7.14 | 6.64 | 0.46 | 3.37 | 2.36 |
| 7 | 7.15 | 6.50 | 0.46 | 4.54 | 2.84 |
| 8 | 7.38 | 6.70 | 0.46 | 4.93 | 3.00 |
| 9 | 7.85 | 7.02 | 0.46 | 5.30 | 3.19 |
| 10 | 8.31 | 7.32 | 0.46 | 6.26 | 3.76 |
| 11 | 8.50 | 7.07 | 0.45 | 7.37 | 4.28 |
| 12 | 8.91 | 7.90 | 0.43 | 6.19 | 3.79 |

* Cream without added acid or alkali.

### *Lipase Content of Milk from Individual Cows*

Representative samples of milk (afternoon milking) were taken from individual cows of the University herd, and were promptly cooled to 3–4° C.

Lipolysis observations were made on (a) sugar-saturated-cream inoculated with the milk in the proportion of 10 to 1 and incubated at 37° C., and (b) the milk samples themselves held at 3–4° C. In both cases lipolysis was followed by the titer of the steam distillate after 0, 24, and 72 hours. Another milk sample was obtained from each of the same cows about a week later and similarly examined. Table 6 gives the results arranged according to breed and the stage of lactation.

TABLE 6

*Lipolysis in milk from individual cows*

| Milk sample, breed, cow no. and mo. of lactation | Titer of steam distillate in cc. N/50 NaOH from | | | | | |
|---|---|---|---|---|---|---|
| | Inoculated substrate at 37° C. | | | Milk itself at 3–4° C. | | |
| | 0 hr. | 24 hr. | 72 hr. | 0 hr. | 24 hr. | 72 hr. |
| Guernsey 470— 2½ mo. | 0.49* | 0.74 | 0.89 | 0.79 | 1.13 | 1.56 |
| | 0.53* | 1.55 | 1.96 | 0.83 | 1.23 | 1.61 |
| " 445— 4 " | 0.50 | 0.70 | 0.84 | 0.64 | 1.16 | 1.62 |
| | 0.53 | 1.56 | 1.88 | 0.68 | 1.19 | 1.57 |
| " 412— 7 " | 0.50 | 1.13 | 1.64 | 0.81 | 1.54 | 2.22 |
| | 0.53 | 1.93 | 2.08 | 0.85 | 2.01 | 2.65 |
| " 427— 7 " | 0.50 | 0.66 | 0.79 | 0.51 | 1.30 | 2.07 |
| | 0.53 | 1.21 | 2.52 | 0.49 | 1.86 | 2.37 |
| " 438—10 " | 0.49 | 2.14 | 3.51 | 0.50 | 2.07 | 3.42 |
| | 0.53 | 3.16 | 4.03 | 0.46 | 2.80 | 3.92 |
| " 432—10 " | 0.48 | 1.91 | 2.23 | 0.47 | 1.74 | 3.04 |
| | 0.54 | 3.24 | 3.91 | 0.49 | 2.99 | 3.84 |
| Holstein 93— 2½ " | 0.49 | 1.25 | 1.93 | 0.57 | 0.92 | 1.24 |
| | 0.47 | 1.27 | 1.97 | 0.52 | 0.94 | 1.32 |
| " 98— 1½ " | 0.49 | 1.32 | 2.01 | 0.64 | 0.91 | 1.18 |
| | 0.48 | 1.71 | 2.25 | 0.56 | 1.27 | 1.94 |
| " 33— 6 " | 0.49 | 1.93 | 3.27 | 0.51 | 2.36 | 3.97 |
| | 0.49 | 2.23 | 3.32 | 0.58 | 2.61 | 3.44 |
| " 67— 7½ " | 0.49 | 1.29 | 2.16 | 0.49 | 1.29 | 2.83 |
| | 0.46 | 1.61 | 2.46 | 0.51 | 2.02 | 2.99 |
| " 91— 8½ " | 0.49 | 1.37 | 3.31 | 0.62 | 1.36 | 3.61 |
| | 0.47 | 2.28 | 3.39 | 0.64 | 2.52 | 3.46 |
| " 83—18½ " | 0.49 | 1.25 | 2.08 | 0.54 | 1.44 | 2.79 |
| | 0.47 | 1.58 | 2.35 | 0.64 | 1.68 | 2.18 |
| Jersey 656— 3 " | 0.47 | 1.93 | 2.39 | 0.58 | 1.33 | 2.01 |
| | 0.44 | 0.59 | 1.23 | 0.46 | 1.39 | 1.83 |
| " 660— 7 " | 0.46 | 1.87 | 2.23 | 0.51 | 1.16 | 1.87 |
| | 0.44 | 0.51 | 1.01 | 0.43 | 1.00 | 1.73 |
| " 621—10 " | 0.47 | 2.10 | 2.61 | 0.63 | 2.40 | 3.94 |
| | 0.44 | 0.59 | 1.40 | 0.49 | 2.02 | 5.09 |
| Br. Swiss 622— 1 " | 0.46 | 1.47 | 2.42 | 0.62 | 1.86 | 3.29 |
| | 0.41 | 1.72 | 2.44 | 0.56 | 1.33 | 4.07 |
| " 809— 6 " | 0.46 | 1.01 | 1.58 | 0.77 | 1.00 | 1.04 |
| | 0.41 | 0.87 | 1.50 | 0.67 | 0.79 | 1.27 |
| " 823—10 " | 0.46 | 1.19 | 1.80 | 0.88 | 1.31 | 2.16 |
| | 0.40 | 1.53 | 1.94 | 0.61 | 1.78 | 2.23 |

* Paired figures do not represent duplicate determinations, but rather two separate determinations on milk samples taken at intervals of about 1 week.

These results indicate appreciable variation in the lipase content or lipolytic activity in the milk from the same cow, as well as wide differences from cow to cow. In several instances the results tend to support the hypothesis that high lipolytic activity accompanies advanced lactation, but taken as a whole the data do not warrant this conclusion. No tests were made in which the lipolytic activity of milk from the same cow was followed throughout the lactation period.

### *Relation of Temperature of Separation to Lipolysis*

To study the temperature of separation as a factor in lipolysis in cream, milk as received commercially was separated at 75° F. and at 110° F., by means of an air-tight separator in such a manner that the fat content of the two creams was alike within 0.5 per cent. Lipolysis was followed in two ways: (a) by inoculating sugar-saturated-cream in the proportion of 10 to 1 with each of the creams and skim milk separately and incubating at 37° C., and (b) by holding the cream at 3–4° C. In both cases lipolysis was measured at 0, 24, 48, and 96 hours by determining the titer of the steam distillate. See table 7.

TABLE 7

*Temperature of separation in relation to lipolysis*

| Description of sample | Acidity of steam distillate in cc. N/50 NaOH after | | | |
|---|---|---|---|---|
| | 0 hr. | 24 hr. | 48 hr. | 96 hr. |
| 75° F. cream in substrate at 37° C. | 0.47 | 1.81 | 2.67 | 3.11 |
| 110° F. cream " " " " | 0.47 | 1.67 | 1.83 | 2.17 |
| 75° F. skim milk " " " " | 0.47 | 2.23 | 2.98 | 3.48 |
| 110° F. skim milk " " " " | 0.47 | 2.10 | 2.40 | 2.74 |
| 75° F. cream at 3–4° C. | 0.89 | 1.60 | 2.66 | 3.00 |
| 110° F. cream at 3–4° C. | 0.78 | 1.24 | 1.81 | 2.01 |
| 75° F. cream in substrate at 37° C. | 0.47 | 2.00 | 3.27 | 4.65 |
| 110° F. cream " " " " | 0.46 | 1.89 | 2.97 | 3.56 |
| 75° F. skim milk " " " " | 0.46 | 2.15 | 3.85 | 4.91 |
| 110° F. skim milk " " " " | 0.46 | 1.93 | 3.04 | 3.88 |
| 75° F. cream at 3–4° C. | 0.75 | 2.99 | 3.26 | 4.01 |
| 110° F. cream at 3–4° C. | 0.68 | 1.73 | 2.33 | 2.92 |
| 75° F. cream in substrate at 37° C. | 0.52 | 1.64 | 2.39 | 3.11 |
| 110° F. cream " " " " | 0.52 | 1.45 | 1.67 | 1.97 |
| 75° F. skim milk " " " " | 0.52 | 1.67 | 2.43 | 3.26 |
| 110° F. skim milk " " " " | 0.51 | 1.54 | 1.79 | 2.03 |
| 75° F. cream at 3–4° C. | 0.76 | 1.19 | 1.87 | 2.16 |
| 110° F. cream at 3–4° C. | 0.71 | 1.06 | 1.24 | 1.43 |

In all three experiments the 110° F. cream showed definitely less lipolysis than the 75° F. cream by both methods. This is in harmony with the findings of Sharp and DeTomasi (9). However, the 110° F. skim milk also showed definitely less lipolysis than the 75° F. skim milk. Since the milk

had been cooled (on farms) before separating, the greater lipolysis in the 75° F. cream when held at 3–4° C. might be attributed to the activation of the lipase by precooling followed by limited warming, a factor as reported by Herrington and Krukovsky (6), but the fact that the same results were obtained when the creams were inoculated into sugar-saturated-cream and incubated at 37° C. argues against this explanation, since 37° C. presumably undoes such activation. The fact that both the 110° F. cream and the 110° F. skim milk caused less lipolysis suggests that partial destruction of the lipase takes place at the higher temperature under the conditions involved in centrifugal separation, or greater loss of lipase in the separator slime (12).

## SUMMARY

1. Formaldehyde addition to sugar-saturated-cream inoculated with raw cream greatly limited lipolysis. Tributyrin greatly increased lipolysis as here measured.

2. Gravity separated cream was found to show appreciably greater lipolytic activity than separator cream of the same fat content.

3. Shaking increased lipolysis in the case of milk samples held as such, but decreased lipolysis in the case of sugar-saturated-cream inoculated with the same milk samples. The latter effect was also observed where cream was used as the inoculum.

4. In a comparison of 3–4° C., 27° C. and 37° C., lipolysis was most extensive at 37° C., slightly less at 27° C. and decidedly less at 3–4° C.

5. The optimum pH for lipolysis in cream samples at 3–4° C. was found to be pH 8.4 to 8.6.

6. The indications are that lipolysis varies considerably in the milk from the same cow, and differs widely from cow to cow. A study of milks from 18 cows at known stages of lactation failed to indicate a relationship between lipolysis and stage of lactation.

7. Cream separated at 110° F. showed less lipolysis than cream separated at 75° F. both as observed by holding the samples at 3–4° C. and by using the cream to inoculate sugar-saturated-cream and incubating at 37° C. Similarly the 110° F. skim milk caused less lipolysis than the 75° F. skim milk in sugar-saturated-cream.

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# THE EFFECT OF HOLDER AND FLASH PASTEURIZATION ON SOME FLAVORS OF MILK. I. THE EFFECT ON MISCELLANEOUS FLAVORS COMMON TO COMMERCIAL RAW MILK[1]

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Feed has long been recognized as a possible contributing factor to the flavor of milk. Early studies on the effects of feeds and feeding practices have aided materially in the establishment of feeding rules which, when followed, result in a minimum of feed flavors in the milk produced. However, much feed-flavored milk is yet produced during certain seasons of the year. Milk distributors often reject feed-flavored milk at the receiving platform on the presumption that if such milk were mixed and processed with the other normal flavor milk, the resulting bottled product would be lowered in flavor quality if not definitely off flavor.

The purpose of this study was to determine the effect of various methods of pasteurization upon the flavor and score of the processed milk as compared to the flavor and score of the control samples. Particularly was information desired on the effects of pasteurization upon some feed flavors in order to determine if those flavors frequently resulting from the feeding of clean wholesome feeds, such as alfalfa and corn silage, were seriously objectionable to the market milk supply.

Several investigators have reported the effects of pasteurization on some flavors of a feedy or barny nature. Tracy and Ruehe (3), holder pasteurizing several deliveries of milk in glass bottles, observed that in practically all cases the barny flavors were partially or completely eliminated by pasteurization, but some feed flavors remained in the processed product. When the milk was heated to 142° F. (61.1° C.) and held for 90 minutes a cooked flavor, which was not apparent at the end of the 30 and 60 minute exposure, was apparent.

Marquardt and Dahlberg (1) found that pasteurization at 143.5° F. (61.9° C.) for 30 minutes not only diminished the intensity of feed flavor but blended the flavors of raw milk to give less variety in the pasteurized product. Usually they found it possible to select raw milk from pasteurized milk by the more pronounced feed flavor in the former.

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Trout and Taylor (4) found that holder pasteurization at 143° F. (61.6° C.) for 30 minutes changed the beet top flavor so that it could not be recognized as such, but did not improve the flavor of the milk to any appreciable extent.

Trout (5) reported finding feed flavors in 23.4 per cent of the samples of raw milk after one day of storage. On the other hand, he did not recognize any feed flavors in the pasteurized samples examined, although a wide variety of other flavors was noted.

Quinn and Burgwald (2) concluded that the high-temperature short-time pasteurization imparted less "cooked" flavor to the milk than did the holder method, but made no comment relative to the elimination of the feed flavors from the milk.

## EXPERIMENTAL

The samples of milk used in this study were secured weekly, from January to June, 1939, inclusive, from ten producers who delivered milk to the College Creamery. Milk from these ten producers were used throughout the study.

Each of the ten weekly samples, divided into four lots, was processed as follows: Lot I, serving as a control, was stored at 40° F.; Lot II was holder pasteurized at 143° F. for 30 minutes in a tightly capped pint milk bottle so as to furnish no aeration during pasteurization and cooling; Lot III was similarly processed, but loosely capped in order to obtain aeration during processing; and Lot IV was pasteurized at 160° F. for 15 seconds by passing the sample through Pyrex glass 7 mm. tubing submerged in hot water and ice baths for appropriate heating and cooling. All samples were stored at 40° F.

After storage for 24 hours part of each sample, for organoleptic examination, was poured into a separate 100 ml. glass beaker and numbered on the bottom according to the key numbers of the samples. The 40 beakers of milk, consisting of raw, of pasteurized unaerated, of pasteurized aerated, and of flash pasteurized samples, were shuffled so that the judge had no knowledge of the sample being examined. After the flavor score and criticism of each sample were recorded the number on the bottom of the beaker was noted and recorded. Immediately following the completion of scoring of the 40 samples, they were reshuffled, rescored and the findings recorded as before, the record being kept on a second paper so the judge had no knowledge of the first score and criticism of the samples. On the third day of storage the samples were again scored and rescored exactly in the same manner as on the first day. Two experienced judges did the scoring throughout. Each judge's score was considered as an observation.

## RESULTS

*Flavors in the raw milk.* A critical study of the samples showed that on the first day of storage 43.3 per cent of the samples were free of flavor criticisms. Of the off-flavors noted, averaging 56.7 per cent, feed flavors predominated with 29.6 per cent; high acid flavors were next with 7.8 per cent; and flat flavors were third with 6.8 per cent. Ten other off flavors, present in a small percentage of the samples, were noted. The distribution of the observations on the samples of raw milk from the ten producers weekly over a six-month period according to flavor is presented in table 1.

TABLE 1

*Distribution of observations according to flavor in milk obtained weekly over a six-month period when pasteurized by various processes and when examined after the first and third days of storage at 40° F.*

| Flavor | Distribution of observations when the milk was | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Raw | | Holder pasteurized, 143° F.—30 min. | | | | Flash pasteurized, 160° F.—15 sec. | |
| | | | Unaerated | | Aerated | | | |
| | 1st day | 3rd day | 1st day | 3rd day | 1st day | 3rd day | 1st day | 3rd day |
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| No criticism | 43.3 | 26.1 | 28.4 | 20.9 | 27.1 | 21.6 | 47.1 | 33.4 |
| Bitter | 0.2 | 0.0 | ..... | 0.2 | ..... | 0.2 | ..... | 0.2 |
| Cooked | ..... | 0.3 | 3.3 | 2.7 | 5.7 | 3.4 | 0.2 | 1.0 |
| Cowy | 2.1 | 0.2 | 0.7 | 0.8 | 1.8 | 0.7 | 1.3 | 0.5 |
| Feed | 29.6 | 21.5 | 19.6 | 12.5 | 16.8 | 10.2 | 19.9 | 15.6 |
| Flat | 6.8 | 5.4 | 5.0 | 3.8 | 6.2 | 6.7 | 6.7 | 5.7 |
| Heated | 2.4 | 3.6 | 34.0 | 30.3 | 29.2 | 25.7 | 13.5 | 12.0 |
| High acid | 7.8 | 21.4 | 2.2 | 2.3 | 2.2 | 1.3 | 2.0 | 2.1 |
| Metallic | 0.3 | 1.0 | 0.2 | 0.8 | 0.2 | 1.3 | 0.2 | 1.1 |
| Off, unidentified | 1.0 | 1.8 | 1.3 | 1.0 | 0.8 | 0.8 | 2.0 | 1.5 |
| Old | 1.8 | 6.0 | 1.3 | 4.8 | 3.7 | 6.7 | 2.6 | 9.4 |
| Oxidized | 1.5 | 4.4 | 2.6 | 18.4 | 4.0 | 18.6 | 2.4 | 13.5 |
| Rancid | 0.2 | 3.4 | ..... | 0.0 | ..... | ..... | 0.2 | 0.6 |
| Salty | 2.8 | 3.3 | 1.2 | 0.8 | 1.8 | 1.5 | 1.3 | 2.8 |
| Unclean | 0.3 | 1.6 | 0.3 | 0.5 | 0.3 | 1.2 | 0.6 | 0.5 |
| No. observations | 616 | 613 | 603 | 598 | 595 | 596 | 613 | 614 |

A study of these same samples after three days' storage at 40° F. showed marked decreases in the number of samples having no flavor criticism and of those having feed criticism. On the other hand, an increase was noted in the number of samples showing off flavors, the major increases being in the number of samples showing high acid, oxidized, and old flavors.

The percentage distribution of the observations on the samples showing specific scores is given in table 2. Here it will be noted that 43.3 per cent of the samples on the first day merited a flavor score of 23. However, by the third day of shortage, the number was reduced to 26.1 per cent of the

TABLE 2

*Distribution of observations according to flavor scores in milk obtained weekly over a six-months period when pasteurized by various processes and when examined after the first and third days of storage at 40° F.*

| Flavor score | Distribution of observations when the milk was | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Raw | | Holder pasteuried, 143 F.—30 min. | | | | Flash pasteurized, 160 F.—15 sec. | |
| | | | Unaerated | | Aerated | | | |
| | 1st day | 3rd day | 1st day | 3rd day | 1st day | 3rd day | 1st day | 3rd day |
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 23 | 43.3 | 26.1 | 28.4 | 20.9 | 27.1 | 21.6 | 47.1 | 33.4 |
| 22 | 22.7 | 17.3 | 41.8 | 37.1 | 36.1 | 31.7 | 29.5 | 22.6 |
| 21 | 20.6 | 24.6 | 23.4 | 22.7 | 27.7 | 22.0 | 17.5 | 24.8 |
| 20 | 5.2 | 9.6 | 4.0 | 9.0 | 5.7 | 9.2 | 3.4 | 11.1 |
| 19 | 3.0 | 4.4 | 1.3 | 5.8 | 1.7 | 9.6 | 1.1 | 5.4 |
| 18 | 4.9 | 15.2 | 1.2 | 4.3 | 1.7 | 5.4 | 1.3 | 2.4 |
| 17 | 0.2 | 1.0 | ..... | 0.0 | ..... | 0.2 | ..... | ..... |
| 16 | ..... | 0.6 | ..... | ..... | ..... | 0.3 | ..... | 0.3 |
| 15 | ..... | 1.1 | ..... | ..... | ..... | ..... | ..... | ..... |
| No. observations | 616 | 613 | 603 | 598 | 595 | 596 | 613 | 614 |
| Mean | 21.83 | 20.92 | 21.88 | 21.45 | 21.76 | 21.28 | 22.14 | 21.59 |
| Standard deviation | ±1.35 | ±1.41 | ±1.07 | ±1.34 | ±1.10 | ±1.50 | ±1.08 | ±1.30 |

samples. The mean flavor score on the first day was $21.83 \pm 1.35$, whereas, on the third day it was $20.92 \pm 1.41$, an average decrease of 0.91 points. This difference was found to be statistically significant.

The general quality of each producer's milk, by months, as indicated by the flavor score is shown in figures 1 and 2. As the summer season approached, there was a general lowering of the score due chiefly to the higher incidence of the feed flavors (figure 3).

*Flavors in the milk that was holder pasteurized without aeration.* A critical study of the samples of milk which were holder pasteurized without aeration showed that 28.4 per cent of the samples had no criticism on the first day of storage (table 1). Of the off flavors noted in 71.6 per cent of the samples, heated flavors predominated with 34.0 per cent, feed flavors were next with 19.6 per cent; and flat flavors were third with 5.0 per cent. Nine other off flavors, present in a small percentage of the samples, were noted. Pasteurization without aeration apparently was instrumental in lowering the frequency of feed flavors from 29.6 per cent in the raw to 19.6 per cent as judged on the first day of storage.

After three days storage there was a decrease in the number of samples having no criticism, or having feed, heated, flat, cooked and off flavors, and an increase in the frequency of old and oxidized flavors. The distribution

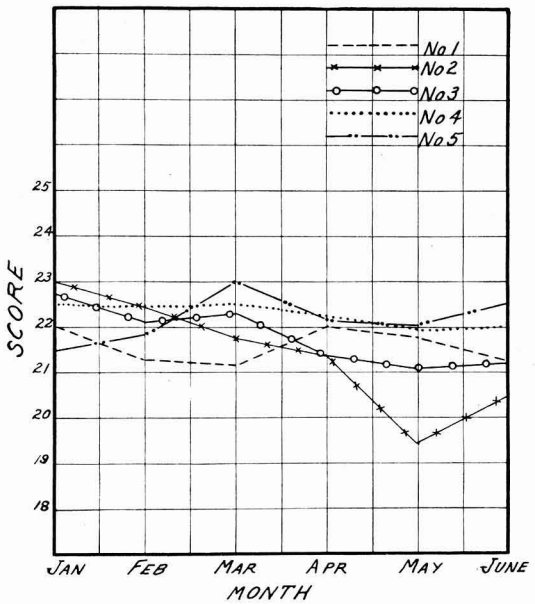


FIG. 1. The flavor score of herd milk from producers 1 to 5 inclusive over a six-months period.

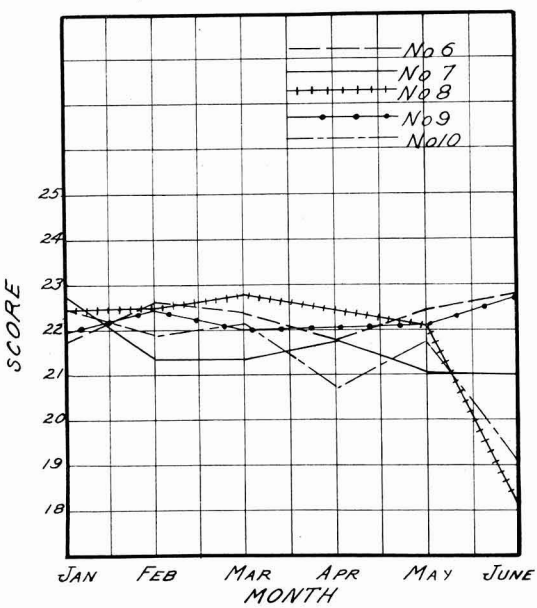


FIG. 2. The flavor score of herd milk from producers 6 to 12 over a six-months period.

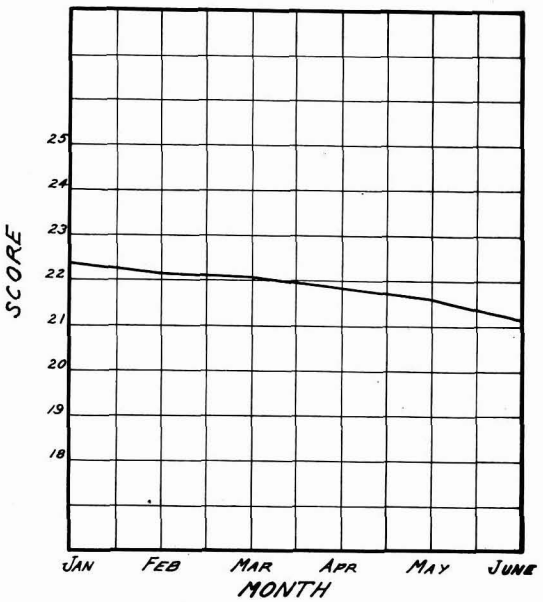


FIG. 3. Mean flavor score of mixed milk from ten producers over a six-months period.

of the samples having other flavors after three days storage remained practically the same as that noted on the first day of storage.

Low temperature holder pasteurization without aeration would seem to be partially effective in eliminating or reducing the intensity of such flavors as feed, high acid, cowy, flat, and rancid. On the other hand such pasteurization resulted in an increase in the frequency of cooked, heated, old, and oxidized flavors.

The mean flavor score of such processed milk on the first day of storage was $21.88 \pm 1.07$, whereas, on the third day the mean flavor score was $21.45 \pm 1.34$ (table 2), a decrease in score of $0.43 \pm 0.07$ which was statistically significant. The mean flavor score of the pasteurized milk samples, first and third day scorings combined, was found to be significantly higher than that of the combined first and third day scores of the raw milk samples. Likewise significant was the increase in score noted in the pasteurized milk over the raw milk after three days storage, but the increase on the first day was not significant.

*Flavors in the milk that was holder pasteurized with aeration.* The results secured by holder pasteurizing with aeration were similar in many respects to those secured in holder pasteurization without aeration (table 1). However, some variations were noted. For example, there was a further decrease in the number of samples showing feed flavors. Likewise, a lower

frequency of the heated flavor was noted. On the other hand, higher percentages of samples showing cooked, flat, and old flavors were observed. However, little difference was found between the unaerated and the aerated samples in the development of the oxidized flavor.

The mean flavor scores on the first and third days of storage were $21.76 \pm 1.10$ and $21.28 \pm 1.50$ respectively (table 2), a decrease in the mean flavor score of $0.48 \pm 0.08$ point after a three-day storage period which was statistically significant. The mean score of the pasteurized aerated samples, first and third day score combined, was slightly higher than that of the raw milk scores combined, the significance of which represented a borderline case. The mean score of the combined first and third day scores of the pasteurized unaerated samples were significantly lower than the mean score of the pasteurized aerated samples. However, the lower scores for the respective days noted in the aerated versus the unaerated samples were not significant in either case.

*Flavors in the milk flash pasteurized.* On the first day of storage, 47.1 per cent of the samples flash pasteurized were free of flavor criticism. This percentage is 3.8 per cent higher than that observed in the control samples; 18.7 per cent higher than in the unaerated holder pasteurized samples, and 20.0 per cent higher than in the aerated holder pasteurized samples.

Of the off-flavors noted in 52.9 per cent of the samples, feed flavors persisted to the extent of 19.9 per cent, which was approximately the same as that noted in the unaerated holder pasteurized samples, but was 3.1 per cent greater than that noted in the aerated holder pasteurized samples. Heated flavors were present in 13.5 per cent of the samples, which is very significantly less than the 34.0 and 29.2 per cent noted in the two respective processes of the holder pasteurized milk. Cooked flavors, noted in a small percentage of the holder pasteurized samples were practically absent in the flash pasteurized samples.

A study of these same samples after storage, showed as, in the other milks, but to a lesser degree, an increase in the frequency of off flavors, 66.6 per cent of the flashed samples being so criticized as compared to 73.9 per cent in the raw, to 79.1 per cent in the unaerated holder pasteurized and to 78.4 per cent in the aerated holder pasteurized samples. Of the flavor criticisms, feed predominated with 15.6 per cent, oxidized with 13.5 per cent, heated third with 12.0 per cent, old fourth with 9.4 per cent, and flat with 5.7 per cent. Nine other off flavors were noted, but these occurred in relatively small percentages of the samples.

The mean flavor scores of the flash pasteurized samples were $22.14 \pm 1.08$ and $21.59 \pm 1.30$ on the first and third days of storage, respectively (table 2), the decrease of $0.55 \pm 0.07$ point being significant.

The mean of the scores on the first day of storage of the flash pasteurized samples was significantly higher than the mean of the raw or holder pas-

teurized samples at similar storage. However, no significant difference was noted between the mean of the flavor scores of the pasteurized unaerated samples and that of the flash pasteurized milk when stored three days, although the difference between the mean scores of the pasteurized aerated and flash pasteurized samples under similar storage was significant. The mean score of any group of pasteurized milk after storage for three days was significantly higher than that of the raw milk samples similarly stored.

Combining the first and third day scores of each group, the means of the flash pasteurized samples were found to be significantly higher than those of the raw, of the unaerated pasteurized, or of the aerated pasteurized milk.

*Flavors tending to disappear or to develop as a result of pasteurization.* The data obtained indicate that two flavors, feed and high acid, tend to disappear markedly with pasteurization. Other flavors decreasing in intensity noted also were cowy, rancid, salty and unclean.

On the other hand the data show that pasteurization favors the appearance of such flavors as heated, cooked and oxidized. Other flavor tending to increase in frequency in the pasteurized samples was old, which is undoubtedly a degree of intensity of the oxidized flavor.

The flat flavors seemed to be current both with raw and with pasteurized

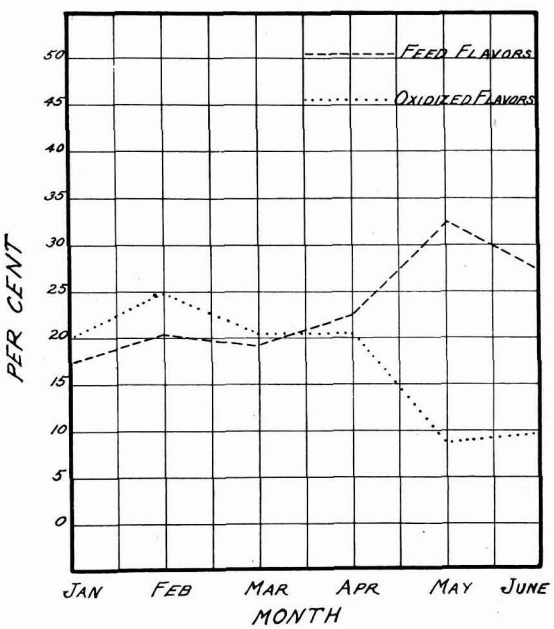


FIG. 4. The distribution of observations as to feed flavor in raw milk over a six-months period and the distribution as to oxidized flavor when the milk was holder pasteurized without aeration, illustrating the relationship noted between the two flavors.

milk, inclining perhaps to be slightly less prevalent in the unaerated holder pasteurized samples.

*The development of the oxidized flavor in the milk during the period of the study.* As previously shown, the oxidized flavor was noted in many of the samples of milk studied. Presented graphically in figure 4 is the percentage distribution of observations showing feed and oxidized flavors. As the frequency of feed flavor increased there was a marked decrease in the frequency of the oxidized flavor. A check on the management of the dairy herds from which the milk in this study was obtained showed that by the third week of April the majority of the producers had turned the cows to pasture, the grass contributing not only the causative agent of the feed flavor but apparently reducing substances inhibiting oxidation of the fatty constituents as well.

This observation on the decreased incidence of oxidized flavors in late spring or early summer is common to general commercial experience. The marked increase of feed flavors which occurred May first was due to the prevalence of grass flavors rather than silage feed flavor.

*Reliability of flavor judgments.* Two experienced judges scored all the samples "blind" and, after reshuffling the samples, rescored them, not knowing the previous score or criticism at the time of the second judgment,

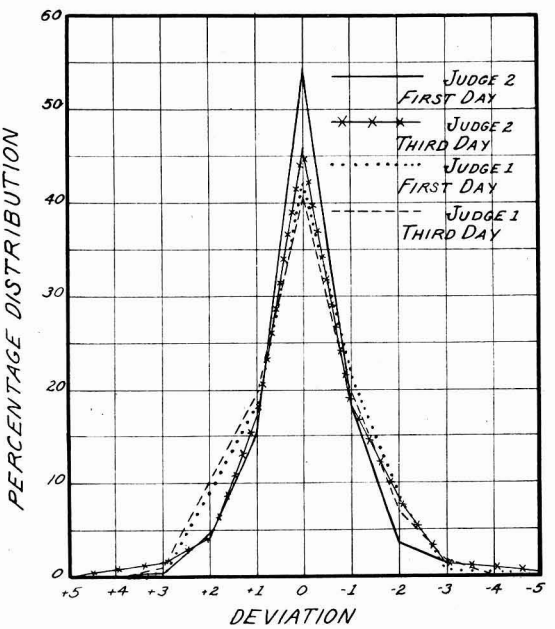


FIG. 5. Distribution curves of the deviation between the first and the second scores of each judge.

in an effort to determine first, the reliability of a single flavor judgment, and second, the closeness of scoring of the two judges. The samples were scored and rescored after the first and again after the third day of storage.

When the milk was scored after one day of storage at 40° F., Judge I rescored 42.0 per cent of the samples identically with the first score (figure 5). He deviated from the first scores on rescoring by $\pm 1$ point in 39.4 per cent of the 596 samples involved; by $\pm 2$ points in 16.9 per cent of the samples; and by $\pm 3$ or more points, the remaining 1.7 per cent. The tendency of this judge was to be more critical and to underscore the samples on second scoring. However, it must be borne in mind that the temperature of the milk rose between first and second judgments; hence, some off flavors might or might not have been detected on the second testing.

After the samples of milk had been in storage for three days they were again scored. The percentage of samples which were rescored identically with the first score was 40.8; those with a deviation of $\pm 1$ were 39.4 per cent; those with a deviation of $\pm 2$ were 17.5 per cent; and those deviating by $\pm 3$ or more points were 2.3 per cent. Judge I had a tendency to rescore the samples higher than the first scores and with a greater range in score. The varying intensities of the flavors which predominated in the milk after three days of storage were such that made accurate rescoring

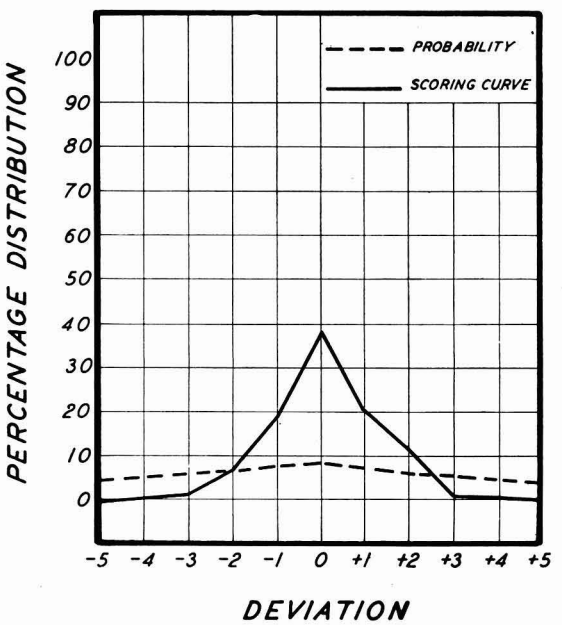


FIG. 6. Distribution curve of the deviation between the judges' scores on all samples of milk as compared to the distribution of probable deviation by random judgment.

difficult. However, little difference was noted in the judges scoring ability in rescoring the fresh or the stored samples.

Judge II rescored 54.3 per cent of the samples of the day-old milk identically with the first score (figure 6). He deviated from his first score on rescoring by a $\pm 1$ point in 33.9 per cent of the 771 samples involved; by $\pm 2$ points in 8.0 per cent of the samples; and by $\pm 3$ or more points in 3.7 per cent of the samples. The tendency of this judge also was to be more critical and to underscore the day-old samples on rescoring.

After the milk was stored three days they were again scored and rescored as before. The per cent of samples which were rescored identically with the first score was 46.8; those with a deviation of $\pm 1$ point was 35.7; those with a deviation of $\pm 2$ was 12.2 per cent and by $\pm 3$ or more points in 5.3 per cent of the samples. Judge II had a tendency to rescore the samples higher and with a still greater range of score than Judge I.

The actual deviation curve between the first scorings of all the samples by the two judges is compared with the probability curve in figure 6. From this the conclusion may be drawn that some factor exists which tends to make the deviation between the judges' scores less than the sampling differences. The possibilities of like agreement by random sampling are so remote that there must be something specific, flavor, in milk which causes the judges not only to recognize its presence but to agree upon its intensity as well.

## SUMMARY

Samples of milk from each of ten producers were secured weekly over a six-month period, divided into lots, were holder pasteurized with and without aeration at 143° F. for 30 minutes and flash pasteurized at 160° F. for 15 seconds. The samples were scored and rescored "blind" at the first and third days of storage by two judges working independently.

The predominating off flavor in the day-old, raw control samples was feed, the percentage frequency of which was materially decreased by pasteurization. The predominating off flavor of unaerated and aerated holder pasteurized day-old milk was heated. Less heated flavors were noted in the flash pasteurized samples than in the holder pasteurized samples. Flash pasteurized milk showed not only a higher percentage of observations of excellent flavor milk, score of 23, than were noted in the raw control or in the holder pasteurized samples but greater stability of excellent flavor upon storage as well.

No significant difference was found between the mean scores of milk holder pasteurized with aeration or similar pasteurization without aeration and that of the raw samples at the first day of storage. However, the increase in score of the pasteurized samples over the raw samples at three days of storage was significant.

Storing the milk for three days at 40° F. resulted in a significant decrease in the score over that noted at one day of storage. Raw milk scores decreased a mean of 0.91 points as a result of storage, whereas, the decrease in score of the pasteurized milk ranged from 0.43 to 0.55 points.

The pasteurization processes increased the frequency of heated, oxidized, cooked, and old flavors and decreased the frequency of feed, high acid, flat, salty, cowy, rancid, unclean, and off but unidentified flavors.

Storage of the milk at 40° F. for three days increased the frequency of high acid, old, oxidized, unclean, and rancid flavors in the raw samples and the oxidized and old flavors in the pasteurized samples, whereas, similar storage decreased the percentage incidence of feed, cowy, flat, heated, cooked, and off but unidentified flavors.

The mean score of the raw milk of all patrons decreased steadily from January through June. A gradual increase in the incidence of feed flavors was found in the raw samples from January to June. During the same period of time there was noted a rather constant frequency of oxidized flavors in the samples of the pasteurized milk, until May when the per cent frequency decreased markedly. As the frequency of feed flavors increased a very similar decrease in the occurrence of the oxidized flavors was noted.

The two judges varied slightly in rescoring ability, the one judge rescoring 42.0 per cent of the samples identically with the first score, whereas, the other judge rescored 54.3 per cent of the samples identically with the first score. Both judges, working independently, scored 39 per cent of all the samples with the same score.

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Milk -- Pasteurization

Milk -- Flavor & odor

# THE EFFECT OF HOLDER AND FLASH PASTEURIZATION ON SOME FLAVORS OF MILK. II. THE EFFECT ON CORN AND ALFALFA SILAGE FLAVORS[1]

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Much feed-flavored milk is delivered daily to milk plants despite the availability of information on feeding management which, if carried out, would prevent the occurrence of such off flavors in the milk. General observation points to the fact that these off flavors are not confined solely to a specific season, although their frequency of occurrence may be greater at some seasons than at others. According to Babcock (1) feed flavors and odors are most frequently caused by succulent feeds. Undoubtedly the winter succulent, silage, plays an important role in the feed flavors of that season. Gamble and Kelly (2) showed that silage flavors were largely imparted to the milk through the body of the cow and were discernible in the milk from cows fed silage one hour before milking. They observed that legume silage fed in equal quantities as corn silage had the more detrimental effect on the flavor of the milk. These silage flavors were found to be partially removable by prompt aeration of the warm milk. They concluded that condensed milk made from silage-tainted milk had a less perceptible silage flavor and odor than the milk from which it was made. Roadhouse and Henderson (4) have recently shown that the feeding of ten pounds of corn silage one hour before milking produced a distinct feed flavor in the milk which was considered undesirable for market milk.

In view of the fact that these feed flavors are often current to milk during the winter season and appear subject to elimination by proper aeration, further studies seemed desirable to determine if such milk pasteurized by the various methods would be acceptable for bottling purposes.

## EXPERIMENTAL

The milk studied in these experiments was obtained from a selected healthy Holstein cow that was about two months in lactation at the beginning of the experiment and giving a good flow of milk. Exactly one hour before milking she was given alfalfa silage or corn silage, as the experiment dictated, in amounts varying from one pound to thirty-five pounds. The

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[2] Dairy Industrial Fellow, 1937.

intensity of feed flavor was given a numerical rating obtained by dividing the pounds of silage fed by the pounds of milk produced. The milk was machine drawn into an aluminum container from which it was poured into a glass bottle which was placed in circulating water for cooling.

The corn silage milk was divided into six lots and treated as follows: Lot I served as a control; Lot II was pasteurized at 143° F. for 30 minutes without aeration; Lot III was similarly heat treated but was aerated by stirring during processing; Lot IV was holder pasteurized also but aerated by passing air up through the milk during the 30 minute holding period; and Lot V was holder pasteurized but subjected to a partial vacuum during processing; and Lot VI was flash pasteurized at 160° F. for 15 seconds and cooled without aeration. A seventh sample, designated as "trap" milk was secured by conducting the exhaust air from Lot IV through a similar volume of cold normal flavor milk, a sample of which was obtained as a control.

The alfalfa silage milk was divided into five lots of which one served as a control. The remaining four lots were holder pasteurized, unaerated and aerated by gentle agitation, by bubbling air through the milk, and by vacuum.

All samples were pasteurized in glass using laboratory equipment. The samples were cooled promptly and stored 24 hours at 40° F. after which they were scored "blind" by two judges. The samples were again scored at three day storage.

## RESULTS

*The relationship between the quantity of corn silage fed and the feed flavor of the resulting milk.* The usual recommendation that a cow may be fed a certain amount of feed one hour before milking without imparting a feed flavor to the milk would appear to be largely dependent upon uniformity of production of milk per cow. As the milk production varies, the volume of flavor in the milk from a given quantity of feed would seem to vary also. Hence, it appears more logical to calculate the pounds of feed fed at a given time per pound of milk produced, in order to ascertain the relationship between the feeding of high flavor feeds and the feed flavor of the resulting milk. Several times during the experiment this assumption was checked by feeding a given quantity of silage to each of a group of individual cows varying in their level of milk production. The intensity of the flavor of the milk varied with the strongest flavor in the milk from the cow producing the least amount of milk. Accordingly, the intensity of the corn silage flavor in the raw control samples in these studies was expressed in terms of the number of pounds of silage fed one hour before milking per pound of milk produced.

Feeding trials showed that the cow could be fed up to 0.67 of a pound of corn silage per pound of milk produced one hour prior to milking without

imparting a feed flavor to the milk. On the other hand, the feeding of approximately 2.00 or more pounds of corn silage under the same conditions per pound of milk produced resulted in a strong feed flavor milk which scored approximately 18.0 to 19.0 on flavor (Table 1).

TABLE 1

*The relationship between the quantity of corn silage fed and the flavor score of the resulting milk*

| Feeding level below 2 pounds per pound of milk produced | | Feeding level above 2 pounds per pound of milk produced | |
|---|---|---|---|
| Pounds of silage per pound of milk | Flavor score of the milk | Pounds of silage per pound of milk | Flavor score of the milk |
| 0.50 | 23.0 | 2.10 | 17.0 |
| 0.67 | 23.0 | 2.34 | 18.5 |
| 0.79 | 20.0 | 2.50 | 18.5 |
| 0.90 | 19.5 | 2.60 | 18.0 |
| 0.94 | 21.5 | 4.00 | 19.0 |
| 0.99 | 18.0 | 4.20 | 21.0 |
| 1.00 | 20.0 | 5.00 | 18.0 |
| 1.05 | 21.0 | 5.10 | 18.0 |
| 1.30 | 19.0 | 6.20 | 18.0 |
| 1.60 | 19.5 | | |
| 1.80 | 19.5 | | |

Higher levels of feeding above a certain point did not seem to have a further detrimental effect on the flavor, probably because of the limited capacity of the cow to consume a given quantity of feed within a given period.

Dilution of several samples of silage flavor milk by normal flavor milk reduced the intensity of the feed flavor to a point where the silage flavor could not be detected. This effect would seem to have practical significance in the grading of milk where but a small percentage of the cans of milk had silage flavor.

*The effect of pasteurization on the flavor of corn silage milk.* Pasteurization had various effects on the removal of the feed flavor resulting from the feeding of corn silage depending upon the type of pasteurization and its modification (Table 2).

Some inconsistencies were noted in the results of holder pasteurization without aeration. In general, this method improved the flavor but the feed flavor was yet distinct. Likewise, holder pasteurization with gentle agitation by stirring or by forcing a current of air through the milk during the holding periods increased the flavor score materially but did not render it free of the distinct feed flavor. However, holder pasteurization under partial vacuum resulted in the removal of practically all the feed flavor. Milk originally scoring around 19 on flavor, when processed by this method usually scored 22 or above.

TABLE 2

*The effect of various methods of pasteurization on the score of corn silage flavor milk after the first day of storage at 40° F.*

| Intensity of silage flavor (lbs. silage/ lbs. milk) | Flavor score when the sample was | | | | | |
|---|---|---|---|---|---|---|
| | Raw | Holder pasteurized, 143° F.–30 min. | | | | Flash pasteurized, 160° F.–15 sec. |
| | | Unaerated | Aerated by | | | |
| | | | Gentle agitation | Bubbling air | Vacuum | |
| 4.00 | 19.0 | ..... | 20.5 | 19.0 | 18.5 | ..... |
| 2.50 | 18.5 | 21.0 | 21.5 | *22.0* | *22.0* | 21.5 |
| 1.80 | 19.5 | *22.0** | 21.5 | 21.5 | *22.5* | *22.5* |
| 1.60 | 19.5 | 19.5 | 21.5 | 21.2 | *22.0* | 21.0 |

* Scores italicized indicate that such milk would likely not be criticized as to taste by the average consumer.

The superiority of partial vacuum holder pasteurization of feed-flavored milk was noted particularly after the milk had been stored for three days. During this period oxidized flavors were prone to develop in the samples holder pasteurized without vacuum, whereas the vacuum pasteurized samples retained the good flavor. This is in agreement with the work of Hand, Guthrie, and Sharp (3) who showed that vacuum cooled milk was less susceptible to oxidative changes.

Flash pasteurization seemed to be on a par with ordinary holder pasteurization in the removal of strong corn silage feed flavor from the milk.

That the corn silage flavor was susceptible to removal by aeration and pasteurization was demonstrated by the fact that gases above the silage milk during processing when conducted through milk, free of feed flavor, imparted a feed flavor to the sample thus treated.

*The effect of pasteurization on the flavor of alfalfa silage milk.* Samples of milk with various intensities of alfalfa silage flavor were holder processed as in the previous experiment. The intensity of the flavor in the original raw milk was again expressed in terms of the pounds of alfalfa silage fed one hour before milking per pound of milk produced.

The alfalfa silage flavor was also volatile and could be transferred, in part, by drawing air through the feed flavor sample into one of excellent flavor, to the extent that a feed flavor could be imparted to the latter.

As in the experiment with corn silage flavor milk, partial vacuum holder pasteurization was again superior to the other modifications of holder pasteurization in yielding a product which was comparatively free from feed flavor and which would merit a score of 22.0 or above on flavor (Table 3). Aeration by bubbling air through the milk during the holding period was partially effective in reducing the alfalfa silage flavor, but practically no difference was noted between the mean flavor scores of the raw and of the pasteurized unaerated samples or of the pasteurized samples aerated by gentle agitation during the holding period.

TABLE 3

*The effect of various methods of pasteurization on the score of alfalfa silage flavor milk after the first day of storage at 40° F.*

| Intensity of silage flavor (lbs. silage/ lbs. milk) | Flavor score when the sample was | | | | |
|---|---|---|---|---|---|
| | Raw | Holder pasteurized, 143° F.–30 min. | | | |
| | | Unaerated | Aerated by | | |
| | | | Gentle agitation | Bubbling air | Vacuum |
| 2.0 | 20.0 | 19.0 | 20.0 | *22.0* | *22.5* |
| 1.6 | 21.0 | *22.0** | 21.0 | ..... | ..... |
| 1.2 | 21.5 | 21.5 | 21.0 | 19.0 | 21.0 |
| 0.73 | 21.5 | 21.0 | 20.0 | 21.5 | *22.0* |
| 0.42 | 18.0 | 21.5 | 21.5 | *22.5* | *22.5* |
| 0.40 | 20.5 | 20.0 | 19.0 | *22.0* | *22.0* |
| 0.22 | *23.0* | 21.5 | 21.5 | *22.0* | *22.0* |
| 0.18 | 21.5 | *22.5* | *22.0* | *23.0* | *22.5* |
| 0.08 | *23.0* | *22.0* | *23.0* | *23.0* | *23.0* |
| Mean ..... | 21.1 | 21.2 | 21.0 | 21.8 | 22.1 |

* Scores italicized indicate that such milk would likely not be criticized as to taste by the average consumer.

A marked difference was noted in the flavors of the milk resulting from the various methods of pasteurization when the milk was stored for three days at 40° F., particularly in respect to pasteurizing under partial vacuum. The partial vacuum pasteurized milk tended to retain its more excellent flavor, whereas different stages of oxidation were noted in many of the samples not vacuum pasteurized.

## SUMMARY

When cows were fed a given quantity of silage the feed flavor was more intense in that milk from the cows of least production. Feed flavors were noted in the milk when 0.79 pounds of corn silage or 0.40 pounds of alfalfa silage per pound of milk produced were fed to the cows one hour before milking.

Alfalfa and corn silage flavors in milk were lessened in intensity by pasteurization. However, strong silage flavors were not entirely eliminated by the processes employed.

Vacuum holder pasteurization and forced aeration holder pasteurization were superior to unaerated or aerated holder pasteurization in removing corn and alfalfa silage flavors from milk. These processes were superior also to flash pasteurization in removing corn silage flavor from milk. Unaerated and aerated holder pasteurization resulted in a greater frequency of oxidized flavors in the stored milk than did vacuum pasteurization.

From these studies it appears that a small quantity of silage flavor milk may not necessarily taint the flavor of a large batch of processed milk. Suf-

ficient excellent flavor milk may be added to the silage milk to reduce the flavor intensity to the extent that pasteurization will remove it entirely.

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# PHYSICAL AND CHEMICAL PROPERTIES OF THE FAT GLOBULES ADSORPTION "MEMBRANE." II. NATURE AND ORIGIN OF SURFACE ACTIVE MATERIALS INVOLVED IN CURD TENSION REDUCTION AND PREVENTION OF RENNET CLOT OF COW'S MILK BY "MEMBRANES" FROM NATURAL AND SYNTHETIC CREAMS*

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*Foreword:* This paper is one of a series giving the results of studies carried out more or less simultaneously in the two laboratories designated in the authorship of this paper. These studies have proceeded with a free exchange of results and frequently of detailed data. The second part of the present paper is a portion of the joint study and contains data obtained in the California laboratory.

## INTRODUCTION

In an earlier paper we (1) showed that reduced or very low curd tensions are exhibited by artificial "buttermilks" produced by churning synthetic creams whose fat globule adsorption "membranes" are derived from (a) washed sweet cream, (b) sweet rennet whey and (c) sols of rennet whey powder. Skim milk containing added natural fat globule "membrane" complex also exhibited reduced curd tension. On the other hand, normal curd tensions were exhibited by "buttermilks" when skim milk was employed as emulsifying agent or when a lecithin-cephalin mixture (80–85 per cent lecithin) was added to skim milk. The conclusion drawn at that time that the fat globule "membrane" itself, particularly its protein component, causes the reduced curd tension of natural buttermilk is supported by our (2) later experiments in which we employed fat globule adsorption "membranes" derived from sols of skim milk powder, calcium caseinate, gelatin, tissue fibrinogen (lecithoprotein) and whey powders (from both rennet and acid whey). When the adsorption "membrane" of the artificial creams was derived from whey powder, gelatin and calcium caseinate, the buttermilk exhibited very low curd tension. When gelatin and calcium caseinate furnished the "membrane," the addition of relatively large amounts of $CaCl_2$ largely, if not completely, prevented the low curd tension

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of the buttermilk but there were indications that this was not due merely to supplying $Ca^{++}$ needed for normal clotting.

The experiments to be reported in the present paper support the conclusion that two somewhat different types of phenomena were involved in our previous results. One type appears to involve a partial denaturation of fat globule "membrane" protein which prevents normal curd tension of the buttermilk, the intermediate steps not yet being clearly understood. In the other type natural esterase in raw milk plasma liberates fat acids which prevent the normal clotting of milk if certain conditions are provided. The application of these findings to natural sweet cream buttermilk will be considered in another paper.

## EXPERIMENTAL

*Procedures and methods.* The general procedures were briefly as follows. Pure, melted butter fat was emulsified at 37° C. in a suitable quantity of the emulsifying agent to produce a synthetic cream containing 27–30 per cent fat. After dilution with skim milk to 3–4 per cent fat content, the "remade" whole milk gave, on centrifugal separation, "remade" skim milk and "remade" cream. The cream, on churning, produced "remade" buttermilk. Any cream which was subjected to washing was diluted with at least four volumes of distilled water in each washing. When it became necessary to concentrate any preparation obtained in this study the concentrate was effected by pervaporation.[1]

The methods for determination of curd tension, surface tension, N content, etc. were described in previous papers (1, 2). In the present study pH was determined in some cases by the hydrogen electrode and in others by the quinhydrone electrode. Any special procedures employed or modifications of the usual ones will be described in connection with their particular use.

1. *Evidence bearing on protein denaturation being involved in the low curd tension of buttermilk.*[2] It is recognized that the term protein denaturation has not yet been defined exactly but it seems to be generally recognized at present that it is the result of intramolecular change, involving the appearance of SH groups, causing a loss of one or more properties, especially that of "solubility." We have previously (1, 2) shown that the curd tension of "remade" buttermilks containing "membrane" proteins derived from whey or whey powder is much lower than when the buttermilks contain natural "membrane" protein. Since whey proteins are definitely

[1] Pervaporation is the term applied by Kober (J. Am. Chem. Soc. 39: 944, 1917) to the spontaneous evaporation of water from colloidal sols through a semipermeable membrane which encloses them. Our method of performing pervaporation is described in a previous paper (1).

[2] The experimental data presented in this section of the paper were obtained at the University of Minnesota and are taken from the thesis presented by N. P. Tarassuk for the Ph.D. degree, University of Minnesota, 1937.

heat coagulable whereas the isolated "membrane" protein shows little if any heat coagulability (3) the possibility is suggested that a reduction in curd tension of a buttermilk may be determined, in part at least, by the extent of denaturation of the "membrane" protein and its release into the buttermilk. It is now established that protein denaturation may be brought about by surface energy as well as by heat. It is certain that surface forces are involved in the formation of an emulsion and in the churning of cream to butter. Also it has been known since the early observation of Ascherson (4) that a denaturation of albumin occurs at an oil-albumin sol interface and that this is accentuated by shaking the mixture.

A discussion of the various prevailing theories regarding the structural and chemical changes occurring in protein denaturation by different forms of energy will not be attempted, but certain results observed in recent studies on surface coagulation of albumin will be cited since like results occurred in the curd tension studies of "remade" buttermilks involving natural whey and whey powder sols. It has been shown by Bull and Neurath (5) that the pH of egg albumin sols shifts during surface denaturation, the direction and extent of shift depending upon the initial pH of the sol. A decrease of 0.2–0.3 pH was found to occur at pH 6.6. In these experiments the rate as well as the extent of surface denaturation at pH 6.6 was relatively low even when other conditions (higher protein concentration and presence of electrolytes) were favorable. Also, they found that even under conditions of maximum coagulation not as much protein could be coagulated by shaking as by heating, although the noncoagulated portion remaining in the heated sample would still show surface denaturation.[3]

If one accepts Neurath's (6) theory that surface denaturation of protein represents an unfolding of the molecule, decreased surface tension should be found to accompany the denaturation. Although this does not seem to have been studied systematically, indirect evidence for it is found in the experiments of Danielli (7) showing that interfacial tension is permanently decreased at an oil-water interface, in the presence of protein.

We admit at the outset that the experimental evidence which we have so far obtained is not as conclusive as we would desire in support of the hypothesis that denaturation of fat globule "membrane" protein may be involved in certain cases of reduced curd tension of buttermilk. The experimental fact that low curd tension is accompanied by lower pH and lower surface tension in the whey protein experiments already published (2) could be accepted as presumptive evidence of protein denaturation when viewed in the light of the foregoing discussion. Since the same effects are produced as the result of milk esterase activity, provided certain fat acids are liberated, as will be shown later in this paper, conclusive evidence for

[3] Protein which has undergone surface denaturation at pH above or below its isoelectric point will coagulate when the pH is brought to this point.

protein denaturation obviously must be sought under conditions which do not suggest esterase activity but where protein denaturation can occur and curd tension is affected.

(a) *Effects on curd tension of direct addition to milk of isolated fat globule "membranes."* If it be assumed that denatured fat globule "membrane" protein may reduce the curd tension of buttermilk when released during the churning of cream, it seems reasonable to expect the same result if the "membrane," isolated from washed cream, is added directly to skim milk. Such a result should be most evident if the "membrane" protein is readily denatured, *e.g.*, in the case of an artificial cream made by dispersing butter fat in a sol whose proteins are exclusively those of milk whey.

Experiments I, II and III, table 1, give the results of tests based on the above hypothesis. In each experiment the principal curd tensions given for comparison are those of (1) the untreated control skim milk, (2) the skim milk plus a "membrane" sol and (3) the skim milk plus water equal to the volume of "membrane" sol tested; the purpose of the last test being to determine the effect on curd tension attributable to the dilution of the skim milk with the "membrane" sol. In experiment II there was an additional comparison in which there was added to the control skim milk a sol of the whey powder having the same concentration of fat and solids-not-fat as in one of the "membrane" sols tested in this experiment. It was thought that this would give additional evidence regarding any change affecting curd tension, which might have occurred in the whey proteins during the preparation and churning of the emulsion.

In experiment III, table 1, an attempt was made to test the possibility that the denatured portion of the "membrane" protein is not confined exclusively to that portion of the "membrane" which remains on the surface of the fat globules after they are washed but is, in part, removed by the washing operation. It is already established (8) that the first washing of cream removes most of the plasma proteins and undoubtedly most of the loosely adsorbed "membrane" also. Regardless of how the denatured portion of "membrane" protein distributes itself in such an experiment, it is safe to say that the first washing plus the free buttermilk from the cream thus washed will contain all of the plasma proteins in the original unwashed cream as well as most of the denatured protein. Some of the "membrane" protein remains in the butter; this portion was not included in our study.

The "membrane" sols tested in experiment III were in one case a concentrate of the first washing of a "remade" whey powder cream and in the other case a mixture of this concentrate and some of the "membrane" from the washed cream. The concentrate of the first washing was added to skim milk when its effect on curd tension was tested alone. When the mixture was tested no plasma proteins were involved except those in the first washing concentrate, the curd tension being determined on a sol made up of nine

parts of this concentrate containing 7.85 per cent s.n.f. and one part of buttermilk concentrate from the washed cream, the latter also containing 7.85 per cent s.n.f.

Since the procedure employed for preparing the several "membrane" sols employed in experiments I, II and III, table 1, differed somewhat, a brief description of each sol seems desirable.

TABLE 1

*Curd tension reductions when artificial fat globule "membranes" are added directly to raw skim milk*

| Sample No. | Description of sample | Curd tension | pH | Surface tension |
|---|---|---|---|---|
| | | *grams* | | *dynes/cm.* |
| | *Experiment I* | | | |
| 1 | Raw skim (blank) | 77 | ..... | ..... |
| 2 | 210 ml. raw skim + 16.9 ml. "membrane" sol No. 1 | 60 | ..... | ..... |
| 3 | 210 ml. raw skim + 16.9 ml. $H_2O$ | 64 | ..... | ..... |
| | *Experiment II* | | | |
| 4 | "Remade" skim (blank) | 52 | ..... | ..... |
| 5 | 90 ml. "remade" skim + 10 ml. "membrane" sol No. 2 | 27 | ..... | ..... |
| 6 | 90 ml. "remade" skim + 10 ml. "membrane" sol No. 3 | 35 | ..... | ..... |
| 7 | 90 ml. "remade" skim + 10 ml. $H_2O$ | 41 | ..... | ..... |
| 8 | 90 ml. "remade" skim + 10 ml. whey powder sol* | 40 | ..... | ..... |
| | *Experiment III* | | | |
| 9 | Raw skim (blank) | 79 | ..... | ..... |
| 10 | 90 ml. raw skim + 10 ml. "membrane" sol No. 4† | 60 | ..... | ..... |
| 11 | 90 ml. raw skim + 10 ml. $H_2O$ | 69 | ..... | ..... |
| 12 | 90 ml. "membrane" sol No. 4 + 10 ml. "membrane" sol No. 5 | 0 | ..... | ..... |
| | *Experiment IV* | | | |
| 13 | Raw skim (blank) | 70 | ..... | 50 |
| 14 | Raw skim + 25% "membrane" sol No. 6 | 36 | 6.53 | 47 |
| 15 | Raw skim + 25% $H_2O$ | 37 | 6.61 | 50 |
| 16 | Raw skim + 14.5% "membrane" sol No. 7 | 53 | 6.46 | 46 |
| 17 | Raw skim + 14.5% $H_2O$ | 53 | 6.62 | 50 |

* This sol had the same concentration of fat and solids-not-fat as "membrane" sol No. 3.

† "Membrane" sol No. 4 had total solids = 11.85%, fat = 4.0%, pH = 6.54.

"Membrane" sol No. 1: A 30 per cent cream was prepared by emulsifying butter fat in nine per cent aqueous dispersion of spray dried rennet whey. The cream was held for 26 hours at 8° C., washed twice[4] at 37° C.,

[4] Chemical analyses of these washings and buttermilk obtained after churning showed 0.035% N in first washing, 0.018% N in the buttermilk and 0.003% N in the second washing.

aged for several hours at 8° C., churned, and the free "buttermilk" concentrated to 3.6 per cent of the original volume. This concentrate constitutes "membrane" sol No. 1.

"Membrane" sol No. 2: This was prepared in the same manner as sol No. 1 except that the synthetic whey powder cream was aged in ice water for four hours and the free "buttermilk" from the twice washed cream was concentrated to only 8.3 per cent of the original volume. The concentrated "buttermilk" constitutes "membrane" sol No. 2.

"Membrane" sol No. 3: A portion of the same 30 per cent cream used for sol No. 2, which had been aged in ice water, was diluted with five volumes of raw skim milk to produce a "remade" whole milk. This was reseparated to produce a "remade" cream. The cream was held overnight at 8° C., washed once at 37° C., churned, and the free "remade buttermilk" concentrated to 6.3 per cent of its original volume. This concentrate constitutes "membrane" sol No. 3. The "remade" skim milk obtained in the course of preparing this sol was employed as the control milk in experiment II.

"Membrane" sol No. 4: A 30 per cent cream in a nine per cent aqueous dispersion of the dried whey was diluted with five volumes of raw skim milk to produce a "remade" whole milk. This was held overnight at 8° C., washed once at 37° C. using four and one-half volumes of water. The first washing was concentrated until it contained 7.85 per cent s.n.f., this being the average s.n.f. content of free "remade" buttermilk from unwashed whey powder cream. This concentrate constitutes "membrane" sol No. 4.

"Membrane" sol No. 5: This preparation is a concentrated sol of the free "buttermilk" from the churned, "remade" washed cream whose first washing was used to produce "membrane" sol No. 4. It was concentrated until it contained the same s.n.f. as "membrane" sol No. 4.

(b) *Evidence that protein denaturation can occur in butter fat surfaces during emulsification and churning.* It was found in the course of our studies that excellent emulsions capable of being churned readily could be produced with butter fat and an aqueous sol of undenatured protein[5] remaining in rennet whey powder after extraction of all lipides by hot 75 per cent ethyl alcohol followed by ethyl ether. These emulsions furnished some evidence of further denaturation as follows:

1. Emulsification. When a concentrated sol of the protein was shaken mechanically in glass for 48 hours at 10°–12° C. both at its original pH (6.35) and at pH 4.85, there was distinct evidence of increased cloudiness

[5] The undenatured, water soluble protein was isolated by a modification of the method described by Rimpila and Palmer (8) for isolating fat globule "membrane" protein. We employed only three volumes of dioxan to one volume of dialyzed, lactose free, filtered protein solution for flocculating the protein, thus permitting the protein to redisperse in water if not subjected to drying. The isolated protein was found to contain only 10.31 per cent nitrogen. The significance of the low nitrogen, which makes it resemble the natural fat globule membrane protein, remains to be discovered.

only at the low pH. However, when the sol was shaken for 30 minutes at 37° C. in the presence of 20 volumes per cent butter fat, and the emulsion centrifuged at high speed, the "skim milk" layer showed evidence of surface denaturation when brought to pH 4.8, especially if the fat emulsion remaining in the lower layer was partially broken by adding one-third volume of ethyl ether.

2. Emulsification and churning. When creams made from butter fat and the protein sol were churned, the buttermilk showed distinct precipitation on acidification to pH 4.0–4.6. It would be expected that such buttermilks should cause a reduction in curd tension when added directly to raw skim milk but this was not found to be the case as shown in experiment IV, table 1. In one test the unaltered buttermilk was employed ("membrane" sol No. 6) and in the other it was first concentrated to 17 per cent of original volume ("membrane" sol No. 7).

### DISCUSSION OF DENATURATION EXPERIMENTS

Experiments II and III furnish the strongest evidence for the possibility of a protein denaturation relationship to reduced curd tension. It should be emphasized that the "membranes" employed in these tests did not remain in contact with milk caseinate for long periods prior to the determination of curd tension so that there was not the opportunity for physico-chemical changes to occur gradually which would prevent the action of rennin. Instead, the "membranes" were added directly to the milk and the curd tension determined almost immediately.

The results show that the effects of "membrane" sols No. 2 and 3, especially No. 2, were clearly greater than obtained by dilution. There is evidence in the same direction, although not in itself convincing, for "membrane" sol No. 1. There is no possibility that lipolytic action could have been involved in the case of "membrane" sol No. 2 for it was not in contact with raw skim milk except at the time of the curd test. Raw skim could have contributed lipolytic enzymes to supply fat acids in the case of "membrane" sol No. 3 but it does not seem likely that it did because "membrane" sol No. 3 had less effect on curd tension than "membrane" sol No. 2. The effect of "membrane" sol No. 4, experiment III (from first washing of a "remade" whey powder cream) was also clearly greater than could be accounted for by dilution and, when combined with the buttermilk from this cream to form "membrane" sol No. 5, did not clot at all when rennet was added. Some of the latter effect admittedly could have been due to lipolysis occurring during the pervaporation of the "remade" buttermilk but it seems unlikely that this was the sole cause since "membrane" sol No. 5 was analogous to "membrane" sol No. 3 which did not completely prevent clot formation.

Special significance possibly should be attached to the curd tension reducing properties of "membrane" sol No. 4 for this represented the outer

"membrane" of the emulsion, removable by washing. The result suggests either that the outer portion of such an adsorption layer is more readily denatured by surface energy or that the portion which has become denatured is more readily detached. The latter view is more in keeping with Neurath's (6) theory of surface denaturation of protein molecules.

It is not possible to explain readily the failure of "membrane" sols No. 6 and 7 to reduce curd tension more than obtained by dilution alone. The stabilizing "membranes" from which these sols were derived were, of course, undenatured residues from the hot alcohol treated whey powder. Although evidence was obtained that they could be denatured further by surface energy, it is possible that not enough further denaturation occurred to affect curd tension. In support of this is the evidence that they had very little effect on either pH or surface tension of raw skim milk.

2. *The effect of esterase activity on curd tension.* The results to be described were the outgrowth of an effort to obtain further light on the question whether a "membrane" protein denaturation is an important factor in the low curd tension of buttermilk. In our previous studies (2) curd tension of buttermilk was either greatly reduced or clotting completely prevented when the membrane was composed of either denaturable proteins (whey proteins) or non-denaturable proteins (casein or gelatin). It seemed desirable to study the effects of employing a "membrane" which was neither protein nor phospholipide, if such could be obtained. We found that diglycol laurate, which is an excellent emulsifying agent, would serve the purpose. The results obtained opened up entirely new aspects of the problem of reduced curd tension and led to the establishment of some of the conditions under which the activity of natural lipolytic enzymes in milk may be responsible for the complete prevention of coagulation by rennet. We will present here only a few crucial experiments among a large number carried out on various aspects of the problem.

(a) *Study of diglycol laurate cream.* A synthetic cream was made by emulsifying 250 ml. fresh, filtered, melted butter fat with 600 ml. six per cent aqueous diglycol laurate suspension at 37° C., using the hand emulsifier, the mixture being put through four times. The emulsion was diluted immediately with 5,720 ml. fresh raw skim milk and the "remade" whole milk separated at once at 38°–40° C. Separation was normal. The remade cream appeared normal after standing overnight in a cold room. Churning at 55°–60° F. required 1.5 hrs., the gathering of the butter granules being slow. The total solids content of the original skim, the "remade" skim and the "remade" buttermilk was 8.5, 8.19 and 8.7 per cent respectively, the fat content of the corresponding products being 0.02, 0.49 and 0.90 per cent respectively. Data on the curd tension with rennet alone at 35° C., the pH and the surface tension are given in table 2, experiment I. All results are averages of at least two determinations.

TABLE 2

*Curd tension at 35° C., pH and surface tension of original and "remade" milks from diglycol laurate cream and of skim milk after direct addition of the same fat acid ester*

| Sample No. | Description of sample | Curd tension | | pH | | Surface tension Fresh |
|---|---|---|---|---|---|---|
| | | Fresh | After aging | Fresh | After aging | |
| | *Experiment I* | *gms.* | *gms.* | | | *dynes/cm.* |
| 1 | Original skim milk | 62 | ..... | 6.60 | ..... | 53.9 |
| 2 | "Remade" whole | 35 | ..... | ..... | ..... | ..... |
| 3 | "Remade" skim | 50 | 49* | 6.58 | ..... | 45.5 |
| 4 | "Remade" buttermilk | 3 | 1* | 6.20 | ..... | 41.3 |
| 5 | "Remade" skim + 30% $H_2O$ | 25 | ..... | ..... | ..... | ..... |
| | *Experiment II* | *After 16 hrs.*† | *After 40 hrs.*† | *After 16 hrs.* | *After 40 hrs.* | *After 16 hrs.* |
| 1 | Original skim milk | 56 | 56 | 6.62 | 6.60 | 53.2 |
| 2 | Skim agitated 30′ at 23° C. | 51 | 54 | 6.66 | 6.60 | 53.2 |
| 3 | Skim + 0.5% diglycol laurate, stirred | 41 | 14 | 6.45 | 6.35 | 35.7 |
| 4 | Skim + 0.5% diglycol laurate, agitated like sample 2 | 0 | 0 | 6.34 | 6.20 | 39.2 |
| 5 | Skim agitated 30′ at 7°–10° C. | 55 | 54 | 6.62 | ..... | 53.2 |
| 6 | Skim + 0.5% diglycol laurate, agitated like sample 5 | 7 | 0 | 6.34 | 6.25 | 36.4 |

* Aging was for 22 hours at 7°–10° C.
† Aging was at 7°–10° C.

(b) *Effect of direct addition of diglycol laurate to skim milk.* Diglycol laurate was emulsified into raw skim milk and the effect on curd tension with rennet alone at 35° C., pH and surface tension studied at intervals. The conditions of the experiment, which had suitable controls, as well as the results are given in table 2, experiment II. Agitation was carried out in a motor driven, four quart Dazey churn having a two blade paddle revolving at approximately 250 r.p.m. All results are averages of at least two determinations.

It is apparent that diglycol laurate cream shows the same phenomenon as synthetic protein creams and that like results are obtained simply by agitating of skim milk containing a dispersion of diglycol laurate. The first explanation which suggests itself is that diglycol laurate is adsorbed by the calcium caseinate, thereby preventing normal rennet action. But this would not of itself explain the simultaneous decline of both curd tension and pH on standing when the ester is added directly to the milk. These effects were repeatedly confirmed. In one of the confirmation experiments the further significant fact was brought out that the use of $CaCl_2$ in the curd test prevented the deleterious effect of the ester on the tension. For example, a

sample of raw skim milk, treated with diglycol laurate, showed a curd tension of 50 grams at 35° C. and a pH value of 6.60 when fresh and a curd tension of zero at 35° C. and a pH value of 6.15 after 40 hours aging at low temperature but the zero value was raised to 61 grams on addition of four volumes per cent of 5 per cent $CaCl_2 \cdot 2H_2O$ solution before addition of the rennet. Analogous effects of $CaCl_2$ were reported in our previous paper (2) when studying the curd tension of "remade" buttermilk from synthetic gelatin and calcium caseinate creams.

The correct explanation of clot prevention in these experiments was found in a study of the cause of the gradual decline in pH. This was found to be due to the liberation of lauric acid from the diglycol laurate ester. The experimental proof will be presented in detail in another paper but experiments I and II, table 3 show clearly that pasteurization of skim milk (30′ @ 65° C.) completely prevents the adverse effect on the rennet clot which results when diglycol laurate is dispersed in raw skim milk.

TABLE 3

*Effect of previous pasteurization on the changes in curd tension, pH and surface tension in agitated, diglycol laurate treated skim milk*

| Sample No. | Description of sample | Curd tension | | pH | | Surface tension |
|---|---|---|---|---|---|---|
| | | A* | B* | A | B | A |
| | *Experiment I* | *gms.* | *gms.* | | | *dynes/cm.* |
| 1 | Past. (blank) | 69 (21) | 62 (44) | ..... | 6.64 | 52.5 |
| 2 | Past. + 0.5% dg.l.,† agitated | 41 | 45 | ..... | 6.68 | 33.6 |
| | *Experiment II* | | | | | |
| 3 | Raw (blank) | 64 (22) | 68 (72) | 6.69 | 6.63 | ..... |
| 4 | Raw + 0.55% dg.l., agitated | 0 | 0 | 6.39 | 6.22 | ..... |
| 5 | Past. + 0.55% dg.l., agitated | 35 | 44 | 6.74 | 6.73 | ..... |

* The figures in parentheses indicate the number of hours the sample was aged at low temperature before the datum was obtained.

† Dg. l. is abbreviation for diglycol laurate.

## SUMMARY AND CONCLUSIONS

Experiments are described showing a reduction in curd tension when artificial fat globule "membrane" sols derived from spray dried whey were added directly to natural or "remade" skim milk. Since some of these sols exhibited evidence of protein denaturation on shaking, the experiments suggest that this may interfere, under some conditions, with normal clotting of milk by rennet.

Experiments are described showing that the normal rennet clot may be completely prevented by emulsifying a small amount of diglycol laurate into raw milk at room temperature, aging the emulsion in the cold and add-

ing rennet at 35° C. This phenomenon also occurs in "remade" buttermilks from "creams" whose butter fat globule "membrane" is diglycol laurate. A decrease in surface tension and pH accompanies the destruction of clotting ability. The explanation of this phenomenon is the liberation of lauric acid from the diglycol ester by natural milk enzyme. It does not occur if the milk is first pasteurized.

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# SPERM STIMULATION IN THE BULL THROUGH THE SUBCUTANEOUS ADMINISTRATION OF ASCORBIC ACID*

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Ascorbic acid therapy for impotency in the male bovine has given rather startling results. The initiation of such treatment was based upon three facts obtained from rather widely separate observations. Phillips and Stare (1) in 1933 reported a very high concentration of ascorbic acid in the pituitary gland of cattle. Phillips *et al.* (2) reported preliminary evidence to show that a low blood plasma ascorbic acid was found in cattle fed on a restricted dietary regimen. Lardy and Phillips (3) found that bulls with low fertility showed less than 2 mg. of ascorbic acid per 100 cc. of fresh semen and in some cases only a trace. Good breeding bulls on the other hand produced semen containing from 3.0–8.0 mg. of ascorbic acid per 100 cc. of fresh semen. With these facts at hand ascorbic acid therapy was undertaken.

Bulls of low potency rating, slowness in breeding and general sexual indifference were sought. Such animals are difficult to obtain because poor breeding bulls are soon dispensed with. A number of such animals have been placed in our hands for treatment. The results obtained seem to warrant publication at this time.

In general, the laboratory data rest largely upon the ascorbic acid content of the blood plasma and semen, the longevity in storage in our yolk buffer pabulum, microscopic observations, and upon return to active breeding service. The ascorbic acid analyses were determined by the method of Mindlin *et al.* (4). Longevity records were obtained by microscopic observations at 37° C. made at 24 hour intervals. Samples thus observed were given ratings of 1+ to 4+ depending upon the type and vigor of the sperm movement. One plus represented poor motility, while 4+ indicated excellent quality semen. The end-point for longevity recording was the change from 1+ to less than 1+ or at the point when only a few sperm were moving.

In all cases herein reported ascorbic acid was injected subcutaneously at the rate of about 1 gram per 1000 pounds of live weight. Such injections

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were made twice weekly where possible. Some cases in the field were given 2 gram doses at five day intervals. Occasionally, it was impossible to give regular periodic treatments where travel and distance were involved. The results obtained indicate that the size of dose should be at least 1 gram per 1000 pounds of live weight. The dose has been increased to 1½ grams per 1000 pounds live weight with good results. Larger doses seem to be unnecessary.

## RESULTS

The results from these studies have been gratifyingly satisfactory. Table 1 shows the progressive recovery of potency by bull No. 1220 under ascorbic acid treatment. It is to be regretted that blood samples were not obtained in this case. It is seen, however, that there was no ascorbic acid in the semen sample obtained prior to treatment. Ascorbic acid began to appear in the semen after 2 or 3 weeks. Thereafter, there was rapid recovery until at the end of 5 weeks the ascorbic acid content of the semen reached the normal

TABLE 1

*The effect of ascorbic acid therapy upon bull 1220,* the first bull treated with ascorbic acid*

| Date | Semen ascorbic acid | Longevity in yolk buffer | Total ascorbic acid injected | Character of semen |
|---|---|---|---|---|
| | *mg. %* | *hr.* | *gm.* | |
| 7/28/39 | 0 | 0 | 0 | Thin, watery, no motility |
| 7/31/39 | ..... | ..... | 1 | ..... |
| 8/ 9/39 | .28 | ..... | 4 | ..... |
| 8/17/39 | trace | ..... | 6 | Thin, feebly active |
| 8/29/39 | 6.88 | 268+++ | 9 | Normal, heavy, highly active |
| Therapy discontinued for three weeks | | | | |
| 9/20/39 | 4.62 | 164+ | ..... | Good, active |
| 10/20/39 | 7.40 | 200+ | 11 | Heavy, highly active |

* A young bull that became sterile shortly after entering service.

level of approximately 6.19 mg. per cent on the average. The appearance of ascorbic acid in the semen was accompanied by distinct changes in the character of the semen sample. Formerly, the semen was thin and watery and totally devoid of life. It had now become normal in appearance, thick and creamy, with every evidence of life and vitality. Ascorbic acid treatment was suspended for 22 days. Tests were again made and 2 grams of ascorbic acid given. Four, seven and eight days later 3 heifers were bred to this bull. One was bred with semen stored for 96 hours. The heifers were slaughtered three weeks after breeding. Embryos were found in two, but the one bred with the stored semen had not conceived.

Table 2 summarizes the data from a number of other bulls given ascorbic acid therapy. Several important facts are noticeable. There was a rise of

95 per cent in the ascorbic acid content of the blood plasma. There was a general movement of the ascorbic acid content of the semen toward a range lying between 3.00–8.00 mg. per 100 cc. of fresh semen. In our experience, completely impotent bulls have shown less than 2 mg. of ascorbic acid per 100 cc. of semen. On the other hand, bulls which had a questionable breeding record, or were only partially potent very often showed excessive amounts of ascorbic acid in the semen sample. There was little change in the ascorbic acid content of successive semen collections taken at one sampling although a slight drop was experienced. With the appearance of normal ascorbic acid values in the blood plasma and semen samples there was uniformly greater sperm vitality as evidenced by the longevity record. This is of special significance.

It will be noticed that the laboratory data on eleven of the bulls was incomplete. These bulls were treated under practical farm conditions. Frequently, herd sires used under natural breeding conditions would not allow semen collections by means of the artificial vagina, were untractable, or distance made it impossible to obtain the data directly. In the latter case veterinarians, county agents, or the authors gave the treatments and obtained reports on the breeding performance as recorded. Where semen samples were available the general characteristics were noted. Semen from seven of these bulls was observed grossly or microscopically, or both. In these cases the semen character was changed by the treatment from the thin aqueous type characteristic of low grade semen to the thick creamy highly motile semen of the normal bull. In this regard it was interesting to record the case of one Guernsey bull which failed to respond to ascorbic acid treatment. It had been noticed that the conversion of carotene might be involved in the impotency of certain bulls. With this observation in mind the above bull was given vitamin A in the form of high grade cod liver oil at the rate of 20 cc. per day. The ascorbic acid therapy was continued. The bull quickly returned to the production of a high grade potent semen which was equivalent to or better than seven other bulls maintained in that particular breeding cooperative association.

Some of the bulls had little sex interest and a few would not breed at all. Six were classified in this category, or were extremely slow breeders. These cases have shown a distinct "lift" in sex interest after therapy. They are again in service.

Two bulls have shown improvement followed by relapse a few weeks after the treatment was suspended. These cases responded again to the therapy after relapse. It seems that the regular heavy service demands upon them crowds them beyond their limits and exogenous sources of ascorbic acid are needed.

An attempt to feed the ascorbic acid by mouth and effect a change in the ascorbic acid content of the blood plasma was unsuccessful. A vicious hard-

TABLE 2

*Partial summary of data on bulls treated by subcutaneous injections of ascorbic acid*

| Bull | Ascorbic acid (mg. %) | | | | Longevity in yolk buffer (hr.) | | Total ascorbic acid administered | Type of case |
|---|---|---|---|---|---|---|---|---|
| | Semen | | Blood plasma | | | | | |
| | Before | After | Before | After | Before | After | | |
| | | | | | | | *gms.* | |
| 121 | 5.36 | 7.44 | ..... | .44 | 0 | 125+++ | 6.0 | Young bull rendered impotent by an infection |
| H | 2.18 | ..... | .22 | ..... | 46+ | 120++ | 2.0 | 5 yr. old show bull |
| 1230 | 4.74 | 5.24 | .18 | .62 | 120+ | 192++ | 6.0 | Young growing bull |
| D | 6.40 | 5.66 | .16 | .36 | 120+ | 240++ | 8.0 | Heavily used mature bull |
| C | 5.20 | 6.20 | .08 | .26 | 96+ | 140++ | 8.0 | " " " " |
| B | 9.70 | 9.00 | .20 | .36 | 24+ | 216+ | 7.0 | Young growing bull |
| V | 7.60 | 6.20 | .22 | .34 | 96+ | 192+ | 16.0 | Mature bull, poor potency |
| Mc | 0.82 | ..... | .30 | ..... | 96+ | ..... | 3.5 | " " " " |
| O | 5.36 | 2.62 | .32 | .50 | 48+ | 216+ | 10.0 | Young mature bull (winter) |
| N | 8.02 | 7.08 | .18 | ..... | 24+ | 192+ | 8.5 | Mature heavily used bull |
| M | ..... | ..... | .20 | .26 | ..... | (15 cows impregnated) | 12.0 | Aged mature bull, lost interest and potency |
| W | 8.10 | ..... | .14 | ..... | 96+ | ..... | 9.0 | Young mature heavily used bull |
| Ave. | 5.77 | 6.18 | .20 | .39 | 69.6+ | 181.5 | 8.0 | |

| Bulls | Before treatment | After treatment |
|---|---|---|
| S, OI, OC, OG, C, B, T, D, W, LP, LA | These bulls showed the following:<br>1. Breeding slow, uncertain<br>2. Semen poor, low motility<br>3. Sex interest indifferent or nil | 1. Breeding improved, positive impregnation percentage increased<br>2. Semen good to excellent, motility active<br>3. Sex interest stimulated and improved<br>4. Returned to service again |

to-handle bull was fed one gram of ascorbic acid daily for 6 days. Subsequent examination of the blood plasma indicated that there had been no change in the ascorbic acid level.

A total of twenty-nine bulls have been treated. Four of these have not responded to ascorbic acid therapy by any measurable means. Therefore, it appears that about four out of every five bulls treated have responded favorably to ascorbic acid injections. One of the bulls which did not respond has been unofficially reported to have developed testicular atrophy previous to treatment. Fifteen of the bulls used in these studies were definitely headed for the discard when they came to our attention. They are again back in regular service after ascorbic acid therapy.

## DISCUSSION

Ascorbic acid therapy is not expected to cure all cases of sterility in the bull. These researches do indicate, however, that it was distinctly beneficial in certain types of sterility. It seems to be favorable when bulls are in the growing and developmental process, or in the cases where rather heavily used potent bulls begin to decline in ability to "settle."

At present it is impossible to suggest why this type of impotency should develop. Ascorbic acid has been shown to be present in the cells of the endocrine glands concerned in reproductive functions. This work has been excellently summarized in the monograph by Giroud (5). Improper feeding over long periods of time, developmental failure, injury or infection might be singly or collectively responsible for this type of sterility. Until the tissues or organs responsible for ascorbic acid synthesis are more clearly defined the cause of impotency due to ascorbic acid deficiency will of necessity remain obscure.

It seems that the ascorbic acid content of fresh semen, freshly drawn blood, and longevity of sperm in yolk buffer gives a fairly accurate rating of potency in the bull. A few samples of semen treated with $H_2S$ and then tested for ascorbic acid under nitrogen gave values very close to those for ascorbic acid alone. Whether there is a relationship between the ascorbic acid and dehydroascorbic acid in semen is not known at this time. In blood plasma certain field samples from outlying points have given abnormally high ascorbic acid values. Whether these values were caused by the liberation of bound ascorbic acid which is released upon standing is not known. This possibility seems unlikely in view of our present knowledge of ascorbic acid chemistry. Longevity of sperm in yolk buffer gives a fair degree of accuracy in rating potency in the bull. This test is easily applicable where semen samples are regularly collected. A poor breeding bull's semen will maintain motility for less than 100 hours while a potent bull semen sample will maintain motility for more than 200 hours. Thus, poor breeding or low potent semen stores for less than 100 hours, good semen will store for 100–

200 hours, and very potent semen will store with a high degree of activity for 200 hours and more.

## SUMMARY AND CONCLUSIONS

These data indicate several important results: (1) the subcutaneous injection of ascorbic acid resulted in the restoration of the fertilizing capacity of certain impotent bulls; (2) potent bull semen normally contained on the average of 6.19 with a range of 3.0–8.0 mg. of ascorbic acid per 100 cc. of fresh semen; values below 2 mg. were associated with impotency, or poor breeding; (3) high ascorbic acid values, 8.0 mg. or more, on the other hand were associated with bulls with an unreliable breeding record; and (4) the ascorbic acid content of fresh semen, freshly drawn blood plasma and especially the longevity of sperm in yolk-buffer provides a fairly accurate estimate of potency or impotency in the bull.

From these data it is concluded that ascorbic acid is intimately involved in the production of virile sperm in the bull and in some manner it is vitally concerned in the physiology of reproduction in the male bovine. The exact nature of its role in this capacity is not known.

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# THE GAS REQUIREMENTS OF MOLDS. II. THE OXYGEN REQUIREMENTS OF *PENICILLIUM ROQUEFORTI* (THREE STRAINS ORIGINALLY ISOLATED FROM BLUE VEINED CHEESE) IN THE PRESENCE OF NITROGEN AS DILUENT AND THE ABSENCE OF CARBON DIOXIDE

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## INTRODUCTION

The object of this study was to determine to what extent the $O_2$ of the air must be diluted with $N_2$ to show the inhibition of growth of strains of *Penicillium roqueforti.*

The previous study (4) has shown the marked difference in the growth of strains of *P. roqueforti* in an atmosphere diluted with $N_2$ as compared with $CO_2$. The study was continued with the object of determining the $O_2$ requirements of strains of *P. roqueforti* in $CO_2$-free air, diluted with $N_2$, over a wide range of temperature. The $CO_2$ in the air and that produced by the mold during growth were absorbed by NaOH solutions, to produce as far as possible the absence of $CO_2$ during growth.

## LITERATURE

Since writing the previous paper (4) of this series, little work has been published on the $O_2$ requirements of any of the molds in the absence of $CO_2$. Certain new work refers to the need for $O_2$ in gluconic acid production by *Aspergillus niger* (6), but since, in the method described, the acid formed is neutralized by precipitated chalk ($CaCO_3$) it is questionable whether the presence of $CO_2$ is not the more important factor, which necessitates a frequent change of air during the fermentation.

## CULTURES

Three cultures of blue mold, strains of *P. roqueforti,* from the previous study (4) were used, namely:

Culture 32. Isolated from a Wensleydale cheese made at the University of British Columbia.

Culture 33. Similar origin.

Culture 37. Isolated from a Wensleydale cheese made by Rowntree, York.

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Studies of their cultural characteristics and morphology have been previously reported (2, 3).

*Purification.* Before commencing the study the cultures were repurified. The usual poured-plate technique was used. The medium employed was very clear whey gelatin, which, with the assistance of a low power binocular microscope, enabled the marking of the colonies soon after germination. When the marked colonies had grown somewhat, they were transferred to agar slants. This was repeated four times. While it was not possible to select individual spores, it is reasonable to conclude that each culture was obtained from the growth of one or more spores of the same origin.

## MATERIALS AND METHODS

*Medium.* From the results in the previous study it was decided to use Difco malt agar. Thus, to prevent changing the medium during the experiment, 17 lbs. of dehydrated Difco malt agar was purchased and stored at approximately 40° F.

*Slants.* Agar slants of the above medium were used for carrying the cultures and also for making the water dispersion of the mold culture to inoculate the plates. When required for water dispersion, the inoculated agar slants were grown for 11 days at from 65° F. to 70° F.

*Preparation of plates.* As in the previous work (4), 25 ml. of the above medium were used for each plate. After pouring, the plates were put on a cold slab to solidify, which prevented condensation on the lids.

*Inoculation of plates.* One ml. of sterile water was added to the 11-day-old slant culture with a sterile pipette, and after mixing it was returned to a 100 ml. water blank and shaken well. A sterile "L"-shaped platinum wire was dipped into the water dispersion of mold spores and used to inoculate the center of the plate. Thus the colony started from a small hole of fairly uniform size made by the platinum "L." Examination under the binocular microscope showed that growth started from an area of 2 mm. or less. The same wire was used in all cases. For each growth curve determination, 5 plates for each culture at each temperature of incubation were inoculated.

*Incubation.* The same battery of incubators was used as in the previous study (4). Seven of these compartments were set to range in temperature at about equal intervals for a total variation of from 46° F. to 90° F. A thermograph or maximum and minimum thermometer was kept in each compartment to determine fluctuations in temperature. Also, 2 thermometers for each compartment were sealed with wax in small bottles containing water and read and recorded twice daily. The average of the readings of the latter was considered as the temperature of the incubator for the period of incubation. The slight variations in temperature from day to day seldom exceeded 1° F. In the later experiments Weston metallic thermometers were fitted into the desiccators and gave the temperature directly in contact with the plates.

*Nitrogen.* In this study, $N_2$ was the only gas used to dilute the air. The $N_2$ was used from the same commercial cylinder of gas.

*Gas chamber.* As in the previous study (4) 250-mm. Pyrex vacuum desiccators were used as the gas chambers for growing the molds in all cases. The maximum capacity of these desiccators is 22 Petri dishes each, when using only the space above the desiccator plate. One desiccator was put in each incubator compartment and connected with 2 glass tubes. One tube was connected to a common gas line to a mercury manometer, a suction pump, and the nitrogen cylinder. The other gas tube was the air intake for each desiccator, which was connected to 4 gas bottles (specially-fitted quart milk bottles). The gas bottles, which were held at the same temperature as the desiccator, were in the following order: First, a bottle of a 10 per cent solution of NaOH to remove $CO_2$ from the air; second, a bottle of dilute solution of $H_2SO_4$ to prevent any NaOH being carried over into the last 2 bottles, which contained a saturated solution of $(NH_4)_2SO_4$ and its crystalline salt; the last bottle was connected to the desiccator. Thus the air drawn into the desiccator passed through NaOH to remove $CO_2$, then through $H_2SO_4$ and finally through a saturated solution of $(NH_4)_2SO_4$ to humidify the air to about 80 per cent and prevent the desiccation of the medium on the plates.

Experimentally, it was found that over a period of 7 days (the 10 per cent added $N_2$ growth curve) a plate lost not more than 1.0 gram at 87° F. or about 0.5 gram at 60° F. To remove $CO_2$ from the air in the desiccator, a porridge dish containing 5 per cent NaOH was held in the space below the desiccator plate.

During the growth period there was no need to move the desiccator from the incubator for changing the gas supply. Thus, variations in changes in temperature were avoided. Furthermore, the desiccator and gas wash bottles were held in their respective incubators before the plates were added, thus shortening the time for adjusting the temperature of the inoculated plates.

*Adding and changing gases.* The desiccators, all having been filled with the required inoculated plates, were simultaneously evacuated to reduce the content of the air to the required fraction. The reduced pressure was measured with the manometer. The $N_2$ was then added to the desiccators until atmospheric pressure was reached.

Example: Required: a mixture of 30 per cent of air and 70 per cent $N_2$. Barometer pressure 700 mm. The desiccators were all simultaneously evacuated to a column of mercury of $\frac{700 \times 70}{100} = 490$ mm. $N_2$ was then added to atmospheric pressure.

A daily change of gas was made simultaneously in all desiccators by bubbling air briskly through the solution in the 4 gas bottles and on through

each desiccator for 10 minutes. Thus, natural air free from $CO_2$ and at the specified humidity was obtained around all the plates in the desiccators. To change the gas over the plates, the desiccators were evacuated to 500 mm. back pressure and returned to atmospheric pressure via the gas bottles. This was repeated in all 3 times. When the molds were required to grow in a gas supply other than air free from $CO_2$, the desiccators were simultaneously evacuated to the required pressure (see above example) and then filled to atmospheric pressure. Both outlet and inlet to the desiccators were then closed; thus, only very slight deviation from atmospheric pressure occurred. The daily gas change took from 40 to 60 minutes. Being repeated 7 times for each growth curve of 168 hours, an unavoidable error of 4 to 5 hours growing in air is introduced in each growth curve.

*Growth period.* After inoculation, which required less than one hour, the plates were inverted and put in their respective desiccators and incubated for a period of 7 days.

*Measurement of colony.* Wherever possible, the growth of the colony of mold was expressed in millimeters representing the average diameter of 5 colonies. (There were very few exceptions where the organisms either failed to grow or became contaminated, in which case the average diameter was obtained from less than 5 colonies.)

*Expression of growth.* From the above measurements, curves have been drawn for each change in gas supply, using millimeters growth as the vertical axis and temperature as the horizontal axis. Such curves have the advantage of permitting:

1. The making of a control curve for all temperatures of growth. Thus, controls do not have to be run concurrently with each curve made under changed gas supply.

2. The interpolation of the average size of colony for any temperature over the range of growth for the culture.

3. By interpolation the plotting of a growth curve for any temperature, having growth as the vertical axis and concentration of any gas at the horizontal axis.

4. The capacity of the culture of mold to grow under a definite condition, to be expressed by the area enclosed by the curve. (The areas were expressed in the same units.)

5. The expression of growth in air, less $CO_2$ on the basis of 100 for all temperatures. Thus a comparison can be made with the growth of the same culture grown under any other gas supply at the same temperature, and in the same medium.

Example: Culture 32 D grown at 70° F. showed from the growth curve by interpolation a diameter of 55 mm. in air less $CO_2$. Culture 32 D grown at 70° F. showed from the growth curve by interpolation a diameter of 48 mm. in air less $CO_2$ 20 per cent, added $N_2$ 80 per cent.

Thus, as $55 : 48 = 100 : x$

$$x = \frac{48 \times 100}{55} = 87.3$$

NOTE: The method permits of seeing at once the percentage of variation on a common basis for all cultures and for any temperature of growth. Comparisons with many other organisms, which are adapted to the technique, can later be made.

Also, insignificant variations (*i.e.*, not exceeding 10 per cent from the control) are immediately apparent.

## PRELIMINARY EXPERIMENT

An experiment was conducted according to the method already described to develop 3 control curves, namely:

1. Air less $CO_2$ (Approximately 21 per cent $O_2$).
2. Air with no $CO_2$ removed (Approximately 21 per cent $O_2$).
3. Air less $CO_2$ evacuated to 90 per cent vacuum to determine whether a high vacuum would effect the mold growth (Approximately 21 per cent $O_2$).

The curves are given in figures 1, 2, and 3 for the cultures 32 D, 33 D, and 37 D respectively; also in table 1. The curves for each culture show that there is not a significant difference of over 10 per cent except for curve 3 which is slightly irregular at high temperatures. The curves 1 (air less

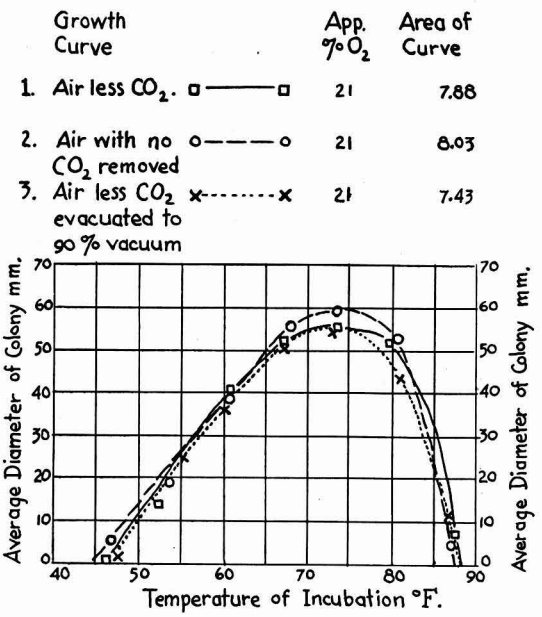


FIG. 1. Control curves to determine the significance of different techniques. Culture 32 D.

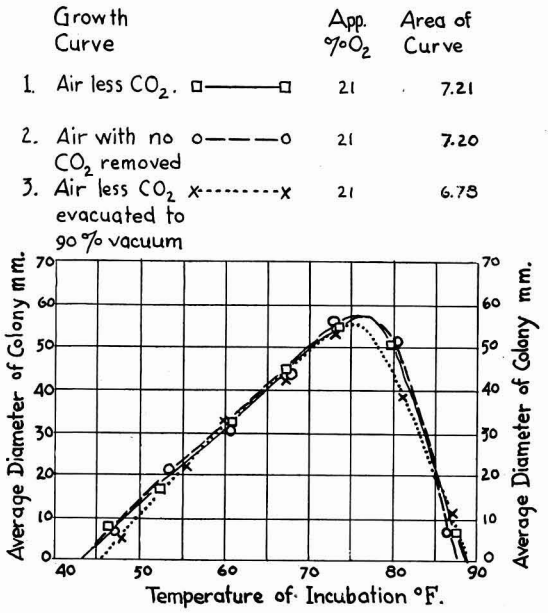


FIG. 2. Control curves to determine the significance of different techniques. Culture 33 D.

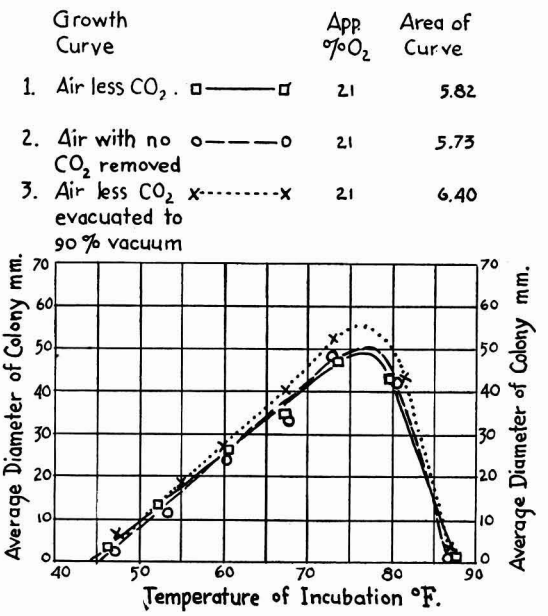


FIG. 3. Control curves to determine the significance of different techniques. Culture 37 D.

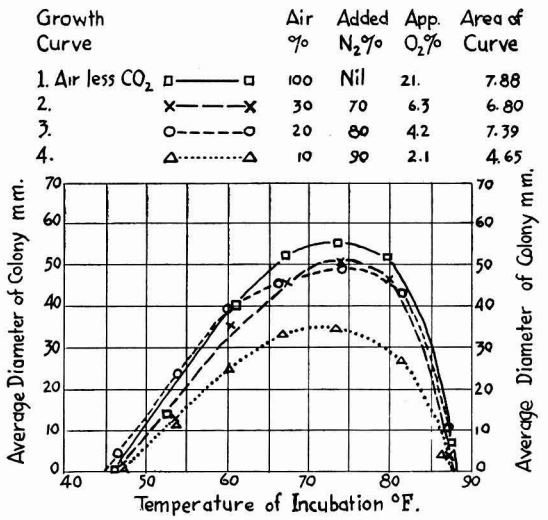


FIG. 4. The effect on the growth of *P. roqueforti* by the reduction of oxygen obtained by adding nitrogen, culture 32 D.

$CO_2$) and 2 (air with no $CO_2$ removed) for all 3 cultures nearly superimpose and would justify using either as control. In all subsequent experiments curve 1 (air less $CO_2$) was used as the control for comparison, with the other curves having a reduced $O_2$ supply in the absence of $CO_2$.

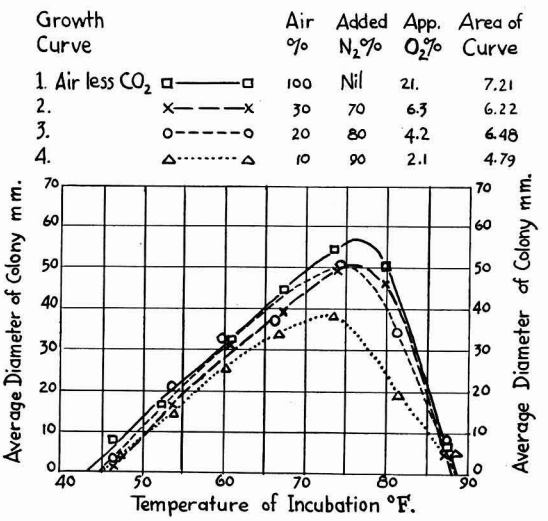


FIG. 5. The effect on the growth of *P. roqueforti* by the reduction of oxygen obtained by adding nitrogen. Culture 33 D.

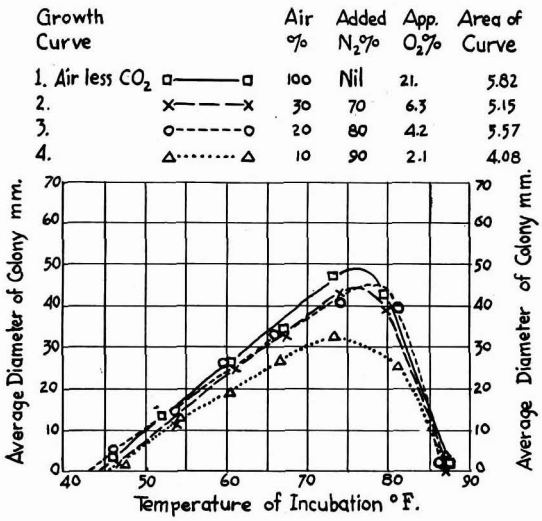


FIG. 6. The effect on the growth of *P. roqueforti* by the reduction of oxygen obtained by adding nitrogen, culture 37 D.

## EXPERIMENTAL

*The oxygen requirements in the presence of $N_2$ as diluent and the absence of $CO_2$*

Figures 4, 5, and 6, growth curves for cultures 32 D, 33 D, and 37 D, respectively, show the seven-day growth curves when the air less $CO_2$ has

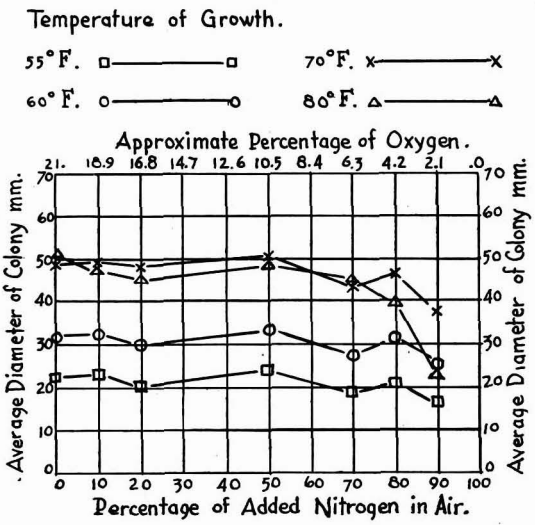


FIG. 7. The effect on the growth of *P. roqueforti* by the reduction of oxygen obtained by adding nitrogen. Culture 33 D.

TABLE 1

*Acceleration or reduction in growth of cultures of P. roqueforti resulting from a change of gas supply. Expressed on the basis of growth in air less $CO_2$ at the same temperature as 100. Seven days' growth*

| Curve | Gas supply by volume | | | Culture | Temperature | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Air % $CO_2$ | Added % $N_2$ | Approximate % $O_2$ | | 55° F. | 60° F. | 70° F. | 80° F. | Area |
| 1 | 100 less $CO_2$ | Nil | 21 | 32 D | 100 | 100 | 100 | 100 | 100 |
| | | | | 33 D | 100 | 100 | 100 | 100 | 100 |
| | | | | 37 D | 100 | 100 | 100 | 100 | 100 |
| 2 | 100 | Nil | 21 | 32 D | 106 | 97 | 105 | 102 | 102 |
| | | | | 33 D | 102 | 103 | 100 | 100 | 100 |
| | | | | 37 D | 91 | 100 | 102 | 102 | 98 |
| 3 | *100 less $CO_2$ | Nil | 21 | 32 D | 96 | 92 | 100 | 90 | 94 |
| | | | | 33 D | 96 | 103 | 100 | 86 | 94 |
| | | | | 37 D | 103 | 108 | 112 | 117 | 110 |

* Evacuated to the same pressure as for 90% $N_2$.

TABLE 2

*Acceleration or reduction in growth of cultures of P. roqueforti resulting from a change of gas supply. Expressed on the basis of growth in air less $CO_2$ at the same temperature as 100. Seven days' growth*

| Curve | Gas supply by volume | | | Culture | Temperature | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Air % (Less $CO_2$) | Added % $N_2$ | Approximate % $O_2$ | | 55° F. | 60° F. | 70° F. | 80° F. | Area |
| 1 | 100 | Nil | 21 | 32 D | 100 | 100 | 100 | 100 | 100 |
| | | | | 33 D | 100 | 100 | 100 | 100 | 100 |
| | | | | 37 D | 100 | 100 | 100 | 100 | 100 |
| | 90 | 10 | 18.9 | 32 D | 108 | 100 | 100 | 100 | 102 |
| | | | | 33 D | 104 | 110 | 102 | 98 | 98 |
| | | | | 37 D | 97 | 104 | 107 | 105 | 103 |
| | 80 | 20 | 16.8 | 32 D | 110 | 103 | 104 | 100 | 103 |
| | | | | 33 D | 89 | 97 | 98 | 92 | 92 |
| | | | | 37 D | 94 | 100 | 100 | 93 | 97 |
| | 50 | 50 | 10.5 | 32 D | 108 | 103 | 98 | 92 | 99 |
| | | | | 33 D | 109 | 110 | 104 | 102 | 105 |
| | | | | 37 D | 100 | 100 | 98 | 98 | 100 |
| 2 | 30 | 70 | 6.3 | 32 D | 84 | 85 | 89 | 88 | 86 |
| | | | | 33 D | 84 | 90 | 90 | 90 | 86 |
| | | | | 37 D | 89 | 92 | 93 | 90 | 88 |
| 3 | 20 | 80 | 4.2 | 32 D | 108 | 102 | 87 | 87 | 94 |
| | | | | 33 D | 96 | 103 | 96 | 80 | 90 |
| | | | | 37 D | 94 | 96 | 90 | 102 | 96 |
| 4 | 10 | 90 | 2.1 | 32 D | 64 | 64 | 64 | 56 | 59 |
| | | | | 33 D | 76 | 84 | 76 | 46 | 66 |
| | | | | 37 D | 72 | 76 | 73 | 64 | 70 |

been diluted with 70, 80, and 90 per cent $N_2$ respectively, as compared with the control air less $CO_2$. The additional seven-day growth curves of the 3 cultures when the air less $CO_2$ has been diluted with 50, 20, and 10 per cent $N_2$ respectively are not given as they superimpose with the control. However, these results obtained from the curves by interpolation are shown in figure 7 for culture 33 D.

Figure 7, for culture 33 D, was plotted by interpolating the points from the seven-day growth curves for temperatures of 55, 60, 70, and 80° F. and show by another expression the effect of reduced oxygen supply by adding $N_2$ in the absence of $CO_2$ on growth.

Table 2 shows the acceleration or reduction in growth of the cultures on a percentage basis, resulting from a reduction in $O_2$ by the addition of $N_2$ to the gas supply.

Together, the growth curves, figures 4, 5, and 6, with growth plotted against concentration of gas, figure 7 and table 2 in which growth is expressed on a percentage basis show:

1. A very low $O_2$ concentration, in the order of less than 4.2 per cent, is required before growth can be significantly reduced.*

2. There is a definite trend for the reduction in growth—caused by low $O_2$, shown in curves 5 and 6—to be proportionately greater above the optimum temperature of growth than that below the optimum.

3. Where temperatures below 55° F. are used, the period of 7-day growth is not sufficient to obtain large enough colonies for a good comparison.

4. The 3 strains of mold used show little difference in their response to the inhibiting effect of very low concentrations of $O_2$. However, it is fairly definite that culture 33 D is most affected above the optimum temperature of growth, while it is probably least affected below optimum temperatures.

## DISCUSSION

The data in this paper agree with the data presented in the previous paper (4) insofar as the work is comparable. The length of the growth period in the previous study (4) was longer at the low temperatures of growth and the methods of adding the gas and handling the controls were sufficiently different to account for the small variations recorded. The data in this paper which indicate that no appreciable effect in growth is shown until very low concentrations of $O_2$ are reached are in agreement with the findings of Brown (1) using *P. glaucum* and *Fusarium* sp.

The reduction in growth of *P. roqueforti*, which is recorded by Thom and Currie (5), cannot in the least be attributed to low concentrations of $O_2$ which they obtained by adding $CO_2$.

* It was observed that the appearance of the colonies was not in the least changed, except in size, between the range of 21 and 2.1 per cent of $O_2$ when $N_2$ was used as the diluent of the air.

It would seem that the reduction of growth brought about by lowering the $O_2$ supply in the presence of $N_2$ first occurred at the high temperatures of growth. The cause of this has not been determined. However, should this be a function of the absorption coefficient of the gas ($O_2$), the problem of whether it is associated with the medium or the moisture content in the mold itself will have to be determined. Whatever the cause, there is no question that this part of the investigation still presents a valuable and interesting physiological study.

## CONCLUSION

1. Three strains of blue mold (*P. roqueforti*) from cheese have been grown at 7 different temperatures in atmospheres of from 21 per cent to 2.1 per cent $O_2$, obtained by adding $N_2$ to the air. The results are expressed graphically.

2. It was only with the greatest $O_2$ dilution (2.1 per cent $O_2$) that a significant reduction of growth was recorded. This ranged between 16 per cent and 54 per cent.

3. It would appear that there is a tendency for this same shortage of $O_2$ to reduce growth more at the higher temperatures.

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# THE GAS REQUIREMENTS OF MOLDS. III. THE EFFECT OF VARIOUS CONCENTRATIONS OF CARBON DIOXIDE ON THE GROWTH OF *PENICILLIUM ROQUEFORTI* (THREE STRAINS ORIGINALLY ISOLATED FROM BLUE-VEINED CHEESE) IN AIR*

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## INTRODUCTION

This study is a continuation of previous studies (6, 7) to determine the significance on the growth of strains of *P. roqueforti* of various concentrations of $CO_2$ in the air at different temperatures. It was considered that the previous study (6) did not fully cover the range of possible dilutions of air with $CO_2$, or the range of the temperatures of growth of the strains of *P. roqueforti* used.

Therefore, the object of this study was to determine to what extent the addition of $CO_2$ to air affects the growth of strains of *P. roqueforti* at various temperatures of growth.

The complementary paper to this study "The oxygen requirements of *Penicillium roqueforti* in the presence of nitrogen as diluent, and the absence of carbon dioxide" (7) reported experiments conducted with practically identical methods. Thus, the results are directly comparable with one another and permit a close estimate of the relative significance of various concentrations of oxygen, nitrogen and carbon dioxide on the growth of *P. roqueforti* at different temperatures.

## LITERATURE

Since the publication of the previous paper (6), no work has been published on the gas requirements of *P. roqueforti.* However, a great deal of work has been done on the effect of gas storage—mainly increased concentrations of $CO_2$—on fruit (1, 3, 4, 5), eggs (8, 9), and meat (9, 10). These workers were chiefly concerned with the practical use of $CO_2$ in gas cold storage, and while several reported reduction in mold growth by gas cold storage as compared with the control, little has been recorded of the species of mold which is inhibited by the various concentrations of $CO_2$ used. With the exception of Eaves (3), who worked with *P. expansum,* no attempt has been made to work with material which was inoculated with a known culture of mold. It would seem that if the inhibition of mold growth by $CO_2$ cold

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storage is to be regularly accomplished, a more systematic approach to the problem must be undertaken. It is hoped that the procedures used in this and previous papers (6, 7) may be of some assistance in this respect.

Acceleration in growth caused by low concentrations of $CO_2$ on the growth of plant tissue has been observed (3, 12). Therefore, it has been considered desirable to include in this investigation the effect on the growth of *P. roqueforti* at various temperatures of low concentrations of $CO_2$ in air.

## MATERIALS AND METHODS

The materials and methods are identical with those of the previous paper (6) except for the following:

*Gas.* Carbon dioxide was used direct from a commercial cylinder of the gas.

*Gas chamber.* The same Pyrex vacuum desiccators were used for these experiments as in the previous work (7). They were operated in the same manner with the exception that no NaOH solutions could be used to remove $CO_2$. Therefore, the first and second gas bottles (specially-fitted quart milk bottles) were filled with very dilute mercuric chloride and a saturated

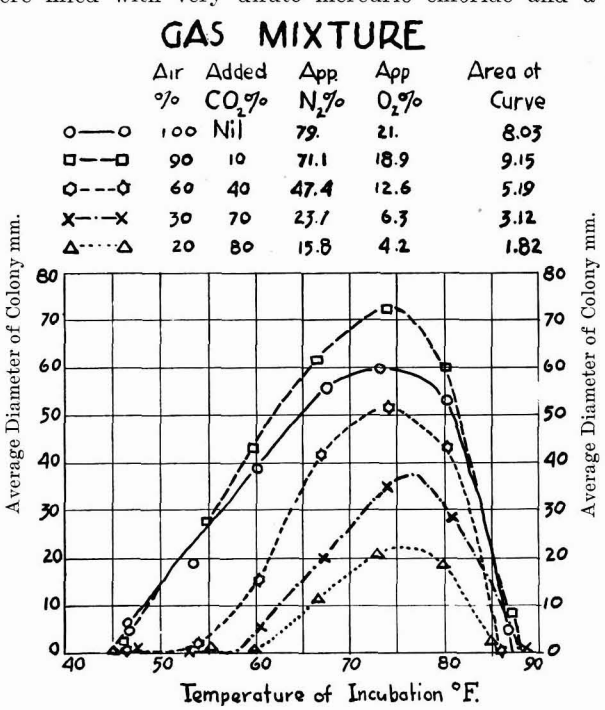


FIG. 1. The effect on the growth of *P. roqueforti* by the reduction of oxygen obtained by adding carbon dioxide, culture 32 D.

$(NH_4)_2SO_4$ and its crystalline salt respectively in place of the NaOH solution and the dilute solution of $H_2SO_4$ previously used. Thus, 4 gas bottles were connected in line so that air entering the desiccator would be maintained at constant humidity and temperature.

*Adding and changing gases.* The method of changing and adding gases to the desiccators was identical with that of the previous study (7) except for the substitution of $CO_2$ for $N_2$.

## EXPERIMENTAL

Five growth curves have been selected and are given in figures 1, 2, and 3 for the 3 cultures used. These curves have been chosen from the 12 curves used in preparing figure 4, and table 1 as they show:

1. The control curve is identical with curve 2 in the previous paper (7) and practically superimposes curve 1 (7) in that paper and was used as the control air less $CO_2$.

2. The maximum increase in growth for each culture, obtained by relatively small additions of $CO_2$, has been selected for each culture on the basis of maximum area, table 1.

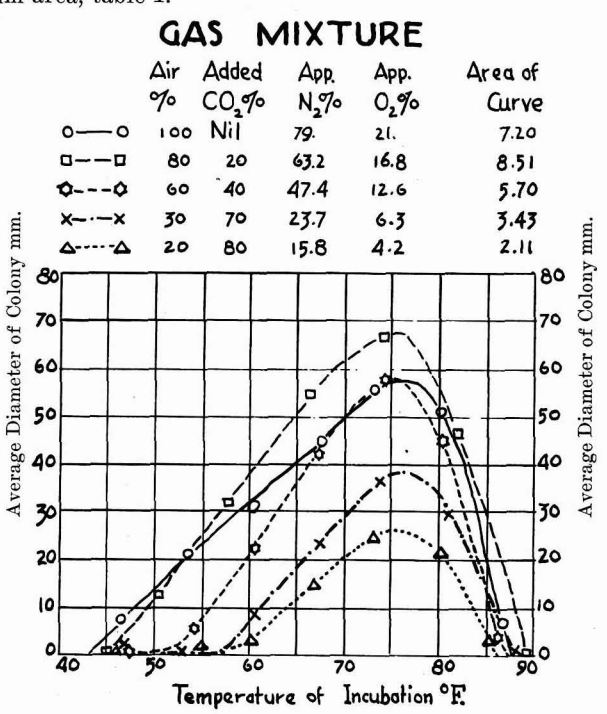


FIG. 2. The effect on the growth of *P. roqueforti* by the reduction of oxygen obtained by adding carbon dioxide, culture 33 D.

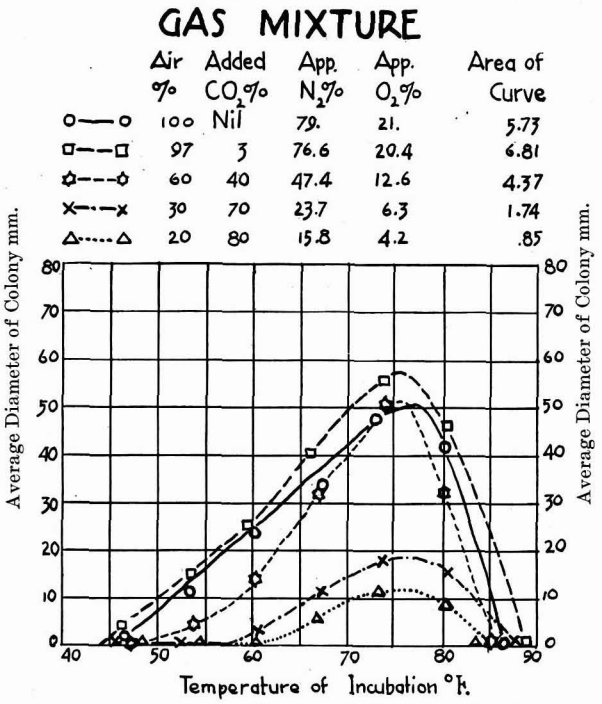


FIG. 3. The effect on the growth of *P. roqueforti* by the reduction of oxygen obtained by adding carbon dioxide, culture 37 D.

3. The remaining 3 curves show the gradual reduction in growth for all 3 cultures by increasing the $CO_2$ content of the air.

Figures 1, 2, and 3, the actual growth curves; figure 4, obtained by interpolation from the 12 growth curves; and table 1, the interpolated results expressed on a percentage basis, each show in their particular way:

1. Dilute concentrations of $CO_2$ in air increase the mold growth.

2. High concentrations of $CO_2$ in air inhibit or even prevent mold growth.

3. The increase or decrease in growth caused by $CO_2$ is a function of temperature. Thus table 1 shows maximum increases in growth at 50° F. for all cultures occurring at 3 or 7 per cent $CO_2$, at 60 and 70° F., maximum growth occurs at 10 per cent or 17 per cent $CO_2$, and at 80° F. maximum growth occurs at 20 per cent or 30 per cent $CO_2$. Figures 1, 2, 3, and 4, show clearly how the inhibition of growth of the molds by $CO_2$ first occurs at the low temperatures and later at the higher temperatures as the concentrations of $CO_2$ are increased. Thus it is seen that at or above 50 per cent $CO_2$ little or no growth can be expected at 50° F., while growth at 60° F. is of the order of 50 per cent.

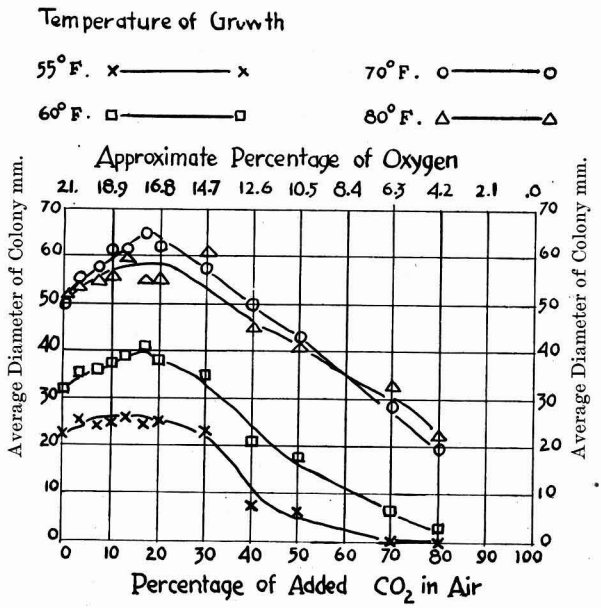


FIG. 4. The effect on the growth of *P. roqueforti* of carbon dioxide in air, culture 33 D.

4. Using the area of the 3 cultures as given in table 1, as an index of their growth response to $CO_2$, it is shown that culture 33 D requires a greater concentration of $CO_2$ for maximum acceleration of growth. Also, the same culture at high concentrations of $CO_2$ is less inhibited in growth by $CO_2$ than the other 2.

## DISCUSSION

This work is in complete agreement with the first paper (6) as far as inhibiting effect of high percentages of $CO_2$ on the mold growth were observed. That $CO_2$ is much more effective in inhibiting mold growth at low temperatures is again shown with more extensive data, which is in agreement with the findings of Brown (2) who used ordinary fruit-rot organisms such as *Botrytis, Fusarium,* and *Alternaria.*

The acceleration of mold growth by small percentages of $CO_2$ is definitely shown. This acceleration of growth is a function of temperature and occurs at the lower temperature of growth before it does at the higher temperatures of growth. Thus 30 per cent $CO_2$ in air shows definite acceleration in growth at 80° F. while the same concentration shows quite definite inhibition at 50° F. Therefore, it is obvious that the optimum and minimum temperatures of growth will be changed by the percentage of $CO_2$ in the gas supply.

Though these general principles apply to all 3 strains of *P. roqueforti,* it is definite that the strains are not equally affected. Culture 33 D is less

TABLE 1

*Acceleration or reduction in growth of cultures of P. roqueforti resulting from a change of gas supply. Expressed on the basis of growth in air at the same temperature as 100. Seven days growth*

| Gas supply by volume | | | | Culture | Temperature | | | | Area |
|---|---|---|---|---|---|---|---|---|---|
| Air % | Added $CO_2$ % | Approximate $N_2$ % | Approximate $O_2$ % | | 50° F. | 60° F. | 70° F. | 80° F. | |
| | | | | 32 D | 100 | 100 | 100 | 100 | 100 |
| 100 | Nil | 79.0 | 21.0 | 33 D | 100 | 100 | 100 | 100 | 100 |
| | | | | 37 D | 100 | 100 | 100 | 100 | 100 |
| | | | | 32 D | *100** | 111 | 114 | 104 | 113 |
| 97 | 3 | 76.6 | 20.4 | 33 D | *107* | 113 | 110 | 108 | 110 |
| | | | | 37 D | 143 | 100 | 119 | 112 | *119* |
| | | | | 32 D | 79 | 105 | 110 | 109 | 106 |
| 93 | 7 | 73.5 | 19.5 | 33 D | 86 | 109 | 116 | 112 | 109 |
| | | | | 37 D | *157* | 100 | 112 | 102 | 110 |
| | | | | 32 D | 100 | *116* | *117* | 115 | *114* |
| 90 | 10 | 71.1 | 18.9 | 33 D | 86 | 116 | 122 | 112 | 116 |
| | | | | 37 D | 129 | 108 | 121 | 102 | 113 |
| | | | | 32 D | 50 | 97 | 110 | 111 | 101 |
| 87 | 13 | 68.7 | 18.3 | 33 D | 86 | 122 | 122 | 120 | 117 |
| | | | | 37 D | 86 | 96 | 114 | 93 | 105 |
| | | | | 32 D | 21 | 105 | 109 | 104 | 98 |
| 83 | 17 | 65.6 | 17.4 | 33 D | 71 | *128* | *130* | 108 | 117 |
| | | | | 37 D | 114 | *112* | *126* | 93 | 117 |
| | | | | 32 D | 71 | 97 | 104 | 106 | 103 |
| 80 | 20 | 63.2 | 16.8 | 33 D | 86 | 122 | 124 | 110 | *118* |
| | | | | 37 D | 100 | 108 | 117 | *116* | 111 |
| | | | | 32 D | 64 | 95 | 107 | *117* | 105 |
| 70 | 30 | 55.3 | 14.7 | 33 D | 71 | 109 | 114 | *124* | 113 |
| | | | | 37 D | 14 | 84 | 107 | 102 | 96 |
| | | | | 32 D | 0 | 37 | 83 | 81 | 65 |
| 60 | 40 | 47.4 | 12.6 | 33 D | 7 | 66 | 100 | 90 | 79 |
| | | | | 37 D | 14 | 52 | 95 | 77 | 76 |
| | | | | 32 D | 0 | 53 | 74 | 85 | 68 |
| 50 | 50 | 39.5 | 10.5 | 33 D | 0 | 56 | 86 | 84 | 73 |
| | | | | 37 D | 0 | 40 | 67 | 58 | 57 |
| | | | | 32 D | 0 | 11 | 45 | 57 | 39 |
| 30 | 70 | 23.7 | 6.3 | 33 D | 0 | 22 | 58 | 66 | 48 |
| | | | | 37 D | 0 | 8 | 33 | 37 | 30 |
| | | | | 32 D | 0 | 3 | 28 | 36 | 23 |
| 20 | 80 | 15.8 | 4.2 | 33 D | 0 | 9 | 40 | 44 | 29 |
| | | | | 37 D | 0 | 0 | 24 | 23 | 15 |

* Italicized numbers indicate greatest acceleration of growth.

quickly accelerated by low concentrations of $CO_2$. The latter is in agreement with the previous work (6).

## DISCUSSION IN RELATION TO THE OXYGEN REQUIREMENTS

Table 1 in the previous paper (7) shows no significant reduction of growth for 4.2 per cent $O_2$ where $N_2$ is the only diluent. The present paper shows a reduction in growth of over 75 per cent for the same concentration of $O_2$ where $CO_2$ is present to the extent of 70 per cent. Thus it is justified to conclude that except in very low concentrations of $O_2$, $CO_2$ was the inhibiting factor and not the lack of $O_2$. The reduction in growth of *P. roqueforti,* which is recorded by Thom and Currie (5), must be entirely attributed to the high concentration of $CO_2$ which was used to dilute the air rather than the low concentration of $O_2$. The effect of temperature shown in the previous paper (7) where $O_2$ and $N_2$ are the only gases present, indicates that the reduction in growth of the molds, which can only be attributed to low concentrations of $O_2$, occurs first at the high temperatures of growth where $O_2$ would be less soluble in the medium. These data show that the effect of $CO_2$ on growth is most noticeable at the low temperature of growth where the $CO_2$ is more soluble in the medium. Neither paper presents sufficient data to justify an attempt at correlation with the absorption coefficient of $O_2$ or $CO_2$ respectively. However, the possibilities of there being such a correlation would seem to be probable.

## CONCLUSIONS

1. Relatively small concentrations of $CO_2$ in air increase the growth of strains of *P. roqueforti* while large concentrations inhibit the growth.

2. With the same organism the acceleration of growth due to low concentrations of $CO_2$ takes place sooner at the lower temperature of growth than at the higher temperature.

3. With the same organism the inhibition of growth, due to large concentrations of $CO_2$ in air, is apparent at the low temperatures sooner than at the higher.

4. The different strains of *P. roqueforti* used show the same trend but have different tolerance to $CO_2$.

5. Culture 33 D was the least affected by the action of $CO_2$.

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# OBSERVATIONS ON THE GROWTH RESPONSES OF STREPTOCOCCUS LACTIS IN MASTITIS MILK[1]

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Several investigators have ascribed certain failures in the manufacturing of cheddar cheese to the presence of mastitis milk. Leitch (1) is of the opinion that mastitis infection should be suspected when recurring difficulties are experienced because of slow acid development, curd weakness and faulty texture. He noted also that the inclusion of even small amounts of mastitis milk in the cheese vat resulted in almost complete suspension of the desired lactic acid development. Whitehead and Cox (2) found that milk containing leucocytes in excess of 5,000,000 per ml. gave rise to a rennet curd in which streptococci were not able to develop normal amounts of acid. Davis and Mattick (3) concluded that visibly abnormal milk should be excluded in the manufacturing of cheese and that milk reacting positively to the strip cup or to the bromcresol-purple test should be regarded as being abnormal until proof is obtained that it may be used with safety. Davis (4, 5) states that slow starter is still the most common fault in cheese making and one of the most serious consequences of mastitis. He is of the opinion that this condition, together with other factors induced by mastitis, causes more trouble than is realized in the making of cheese. According to Davis (6) the most common cause of slow starters in England appears to be abnormal milk from mastitis afflicted cows. He cites four changes in the milk that may influence the rate of growth of starter organisms, namely: (a) changes in the chemical composition of the milk, particularly a decrease in the lactose, casein, calcium and acidity; (b) changes in some enzymes and decreases in some vitamins and bacterial growth factors; (c) increased number of bacteria in the udder; and (d) apparent production, in rare instances, of substances strongly toxic to starter organisms.

Davis and McClemont (7) studied the acid coagulating time in both normal and mastitis milk. With the majority of the mastitis milk samples slow growth of *S. lactis* and *S. cremoris* occurred, whereas most of the normal samples, but not all, supported normal growth of these organisms. They consider that the reason for the slow growth of these organisms, probably, is due to the abnormal chemical composition of such milk.

In the present study frequent observations, over an extended period of time, were made on the growth responses of *Streptococcus lactis* in milk drawn from the individual infected and non-infected udder-quarters of the

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same cows. The infection of some of these udder-quarters was of such a nature that the milk was definitely abnormal at some observation periods and apparently normal at other times.

The experimental data presented include only a portion of that collected, being confined to a study of the milk from three cows, but they are representative of the findings with milk from other animals.

## PROCEDURE

Samples were collected from the individual udder quarters in such a manner as to exclude gross contamination, and each consisted of approximately 200 ml. of milk drawn after the first few streams had been discarded, except in the case of several of the very abnormal milks where it was not possible to obtain this amount at a sampling period. Portions of the milk were inoculated with a culture of *S. lactis,* incubated at 18° to 20° C. and observations made on subsequent growth. The rate of growth was measured by the changes in pH value using the quinhydrone electrode supplemented by microscopic examinations of stained preparations.

## EXPERIMENTAL RESULTS

Of the three cows used in this study, cow 114 gave milk from one udder-quarter that was abnormal at all times, whereas the milk from the diseased udder-quarters of cows 126 and 118, as judged by its pH values and physical state, fluctuated between an apparently normal state and a definitely abnormal condition throughout the period of observation.

A summary of the results, as measured by changes in pH values, of numerous series of milk from each animal is shown in table 1.

TABLE 1

*Range in pH values of milk from diseased and normal quarters before and after inoculation with S. lactis*

| Cow | Quarter | Condition of quarter | No. of samples | Range in pH | | Range in pH after 24 hrs. incubation | |
|---|---|---|---|---|---|---|---|
| | | | | Minimum | Maximum | Minimum | Maximum |
| 114 | LF | Diseased | 14 | 7.10 | 7.67 | 6.44 | 7.30 |
| | RF–RH and LH | Normal | | 6.54 | 6.78 | 4.41 | 5.23 |
| 126 | RF | Diseased | 20 | 6.54 | 7.30 | 4.54 | 7.05 |
| | LF | Diseased | | 6.55 | 7.47 | 4.53 | 7.35 |
| | RH and LH | Normal | | 6.49 | 6.78 | 4.52 | 5.14 |
| 118 | RF | Diseased | 22 | 6.68 | 7.30 | 4.68 | 7.10 |
| | RH | Diseased | | 6.66 | 7.05 | 4.70 | 7.04 |
| | LH and LF | Normal | | 6.49 | 6.82 | 4.30 | 5.10 |

*Cow 114.* The left front udder-quarter was infected with a streptococcus having beta hemolytic characteristics. No attempt was made to

further identify the organism. Milk from this quarter was alkaline in reaction at all times, and ranged in pH values from 7.10 to 7.67. Milk from the remaining quarters ranged in pH value from 6.54 to 6.78. The milk from the left front quarter, when inoculated with *S. lactis* and incubated for 24 hours showed pH values ranging from 6.44 to 7.30 as compared to pH values ranging from 4.41 to 5.23 for milk samples from the normal quarters held under similar conditions of incubation.

Microscopic examinations showed definitely that the milk from the left front quarter of this cow retarded the growth of *S. lactis*, thus confirming the findings as indicated by the study of the pH values.

*Cow 126.* Mastitis of a staphylococcic nature was present in the right front and left front quarters of this cow. Milk from the right front quarter ranged in pH value from 6.54 to 7.30 and milk from the left front quarter from 6.55 to 7.47. Milk from the two apparently normal quarters ranged from 6.49 to 6.78 in pH value. When inoculated with *S. lactis* and incubated for 24 hours the pH values of the samples from the right front quarter ranged from 4.54 to 7.05, for the left front quarter from 4.53 to 7.35 and from the two remaining and apparently normal quarters from 4.52 to 5.14.

The milk samples in which normal acid development did not occur had initial pH values of 6.9 and higher. Two samples, however, with pH values of 6.92 permitted *S. lactis* to develop in a normal manner as judged by decreased pH values.

*Cow 118.* The right front and right hind quarters of this cow showed definite evidence of mastitis due either to a staphylococcus or to an organism having the morphological characteristics of organisms of the genus *Corynebacterium* or to both, since these organisms were present in considerable numbers in both diseased quarters. The milk from these quarters ranged in initial pH values from 6.68 to 7.30 and from 6.66 to 7.05 respectively. The pH values recorded for the milk from the two remaining quarters varied from 6.49 to 6.82. After inoculation with *S. lactis* and incubating for 24 hours, milk from the right front quarter ranged in pH value from 4.68 to 7.10, from the right hind quarter from 5.70 to 7.04 and from the two apparently normal quarters from 4.30 to 5.10. Greater variation occurred with the milk from this cow in the initial pH value of the samples showing retarded acid development than occurred in the milk from cow 126. One sample with an initial pH value as low as 6.68 showed marked delayed acid development, whereas other samples with initial pH values up to 6.95 showed little or no delayed acid development.

### *Effect of adjusting the pH value of mastitis milk to that of normal milk on the subsequent growth responses of S. lactis*

Since the most favorable reaction for the growth of *S. lactis* corresponds to that of normal milk, the relatively high pH values of mastitis milk tend

to create an unfavorable environmental condition for the best development of this organism. Also, a considerable amount of physiological activity is necessary on the part of the culture, in producing an amount of lactic acid sufficient to reduce the pH value of mastitis milk to that of normal milk. Numerous experiments, therefore, were carried out in which the pH value of the mastitis milk was adjusted to that of the normal milk from the healthy udder-quarters by the addition of lactic acid. Both the normal milk samples and the adjusted mastitis milk samples were inoculated with *S. lactis* and observations made in the usual manner.

On incubation the pH values of the adjusted mastitis milk, in almost every instance, failed to decrease as rapidly as did the pH values of the normal milk. Usually there was little or no appreciable change in the pH values of the adjusted mastitis milk during the first fifteen to twenty hours, after which time fairly rapid acid development took place with the final pH values dropping to approximately the same levels as those of the normal milk samples.

### *Effect of mixing normal and mastitis milk on the subsequent growth of S. lactis*

Numerous experiments were carried out in which varying proportions of normal and mastitis milk were mixed, inoculated with the test culture and frequent observations made during the incubating period.

The extent of the retarding effect depended on the degree of abnormality. Many of the samples of definitely abnormal milk in which the pH values were 7.2 and higher exerted a retarding effect upon the growth of *S. lactis* when mixed with normal milk in as little as 10 per cent concentrations. Other samples in which the growth of *S. lactis* was only slightly retarded, lost this characteristic when diluted with as little as 10 per cent of normal milk.

### *Effect of pasteurizing mastitis milk on the subsequent growth responses of S. lactis*

Since it has been shown by Hammer and Baker (8) that the growth of starter organisms is more rapid in milk that has been subjected to high pasteurizing temperatures, numerous experiments were carried out in which portions of mastitis milk were subjected to temperatures of 62.5, 65.5 and 68.5° C. for periods of 30 minutes. Pasteurizing at 62.5° C. had little or no effect on the subsequent growth of *S. lactis*. Pasteurizing some samples at 65.5–68.5° C. for 30 minutes tended to partially overcome the retarding influence on the growth of *S. lactis*. With most of the milk samples, however, heat treatment at these temperatures had little or no appreciable effect.

## DISCUSSION OF RESULTS

With the milks used in this study the retarding effects on the growth of *S. lactis* cannot be attributed to the high pH values nor to the presence of a thermolabile inhibiting substance such as that associated with excessive numbers of leucocytes. Although leucocyte counts were not recorded in this study, the samples that were definitely abnormal contained excessive numbers of these cells as indicated by the examination of stained preparations for the presence of *S. lactis*. In this respect the results reported herein differ from those of Whitehead and Cox (2) in which the inhibiting effect of abnormal milk containing large numbers of leucocytes was entirely removed by heating the milk for 30 minutes at 63° C. The results of this study tend to bear out the contention of Davis and McClemont (7) that the slow growth of *S. lactis* in mastitis milk probably is associated with the changed chemical composition of such milk.

While the extent of the retarding effect of mastitis milk usually was associated with the degree of abnormality such, however, was not always the case. Under the prevailing conditions of milk production it is unlikely that any considerable quantities of definitely physically abnormal milk would find its way into that used for commercial purposes. Certain occasions might arise, however, where milk, that appears normal in its physical properties yet possessing definite inhibiting action on the growth of *S. lactis,* may be present in sufficient amounts to interfere with manufacturing processses dependent upon the development of this organism.

The variations noted in the growth responses of *S. lactis* in the milks from the three cows used in this study may have been due in part, to the respective types of mastitis involved. Unfortunately, no milk for extensive study was available from a cow suffering with *Streptococcus agalactiae* type of mastitis, the type most common among dairy cows. However, in connection with this study, numerous observations were made using milk from *S. agalactiae* infected udder-quarters with the same general results.

## SUMMARY

The growth responses of *S. lactis,* as judged by changes in the pH values and microscopic examinations have been studied in separate samples of milk drawn from the infected and non-infected udder-quarters of cows suffering with mastitis.

Milk with an initial pH value greater than 6.9 usually failed to support the growth of *S. lactis* in an active manner, whereas normal milk from the other udder-quarters showed normal acid development. The growth responses of *S. lactis* varied somewhat in the milk drawn from the three cows under study.

Adjusting the pH value of mastitis milk to that of normal milk resulted in only partially overcoming delayed acid development. Mastitis milk treated in this manner changed little in pH value until after fifteen to twenty hours, after which time acid development was quite rapid.

The addition of as little as 10 per cent of very abnormal mastitis milk to normal milk had a retarding effect on *S. lactis* development.

Pasteurizing some samples of mastitis milk at 65.5° C.–68.5° C. for 30 minutes tended to partially overcome the retarding influence on the growth of *S. lactis;* however, with most of the samples studied heat treatment at these temperatures had little or no appreciable effect.

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# THE RELATIONSHIP OF MOISTURE IN SWISS CHEESE TO QUALITY AND YIELD

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The amount of moisture present is known to be important in the ripening of cheese. Sammis and Germain (11) have shown that, in Cheddar cheese, ripening is influenced by the ratio of moisture to solids-not-fat, and they pointed out that bacterial growth and chemical changes are more rapid in high-moisture cheese than in cheese that contains relatively less moisture. Van Slyke and Hart (13) showed that, in Cheddar cheese, an increase in the percentage of moisture "favors active chemical changes in the process of ripening," and attributed the increased ripening to the effect of moisture in diluting the products of fermentation and increasing the activity of bacteria and enzymes.

Excepting papers in Swiss journals describing work of Koestler (7), Dorner and Stähli (4), and Orla-Jensen (9), very little has been reported on the control of moisture in Emmentaler or Swiss cheese and the effect of moisture content on quality. Koestler showed that a finely-harped curd tends to retain moisture, and that the presence of cheese dust tends particularly to cause high moisture content, leading to excessive fermentation, oversetting, and generally defective ripening. Dorner and Stähli expressed the belief that an insufficient rate and amount of drainage is a common cause of defects, and that when high moisture content results in excessive final acidity, the low pH (high acidity) prevents the formation of normal eyes and causes the curd to be firm and "short" so that cracks appear instead of eyes during the ripening. A similar observation has been referred to in a previous publication (2) from these laboratories. Orla-Jensen found that the use of high cooking temperatures tends to dry the cheese curd in the kettle, but at the same time results in an inhibition of the ripening and a decrease in the rate of eye formation.

The following data on the percentage of moisture in normal Emmentaler cheese are taken from foreign literature. For green cheese: In 18 cheeses (averaging approximately 50 per cent fat in dry matter), average percentage of moisture, 37.08 (7); in 1 cheese, 43.99 (15); in 1 cheese, 40.92 (1); average of 20 green cheeses, 37.62 per cent moisture. For cured cheese:

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[2] The work on factory cheese described herein was conducted with the cooperation of the Departments of Dairy Industry of the University of Wisconsin and the Ohio State University.

In 4 cheeses, 34.71 (15); in 5 cheeses, 35.78 (1); in 4 cheeses, 36.05 (8); in 259 cheeses, 35.19 (12); in 14 cheeses, 35.34 (7); average of 286 cured cheeses, 35.21 per cent moisture. Winkler (16), in a discussion of this subject, stated that 33 to 35 per cent is a desirable range of moisture content, and that 38.2 per cent ought to be the upper limit. Orla-Jensen (9) has given values between 35 and 36.5 per cent as being representative for cured Emmentaler cheese.

Comparison of a large amount of data obtained in analyzing domestic Swiss cheese with the data cited above indicates that, even though Emmentaler cheese is cured longer than domestic Swiss cheese, much of our Swiss cheese is abnormally high in moisture content in comparison with Emmentaler cheese of similar age. There is a belief in some quarters, expressed particularly by cheese dealers, that the practice of incorporating excessive amounts of moisture in this and certain other varieties of cheese is detrimental to quality. On the other hand, it is commonly believed that the presence of a high percentage of moisture is accompanied by a proportionate increase in yield, although no data on this subject have been published.

The purpose of this paper is to present a report of information collected in a study of some of the factors causing variations in the amount of moisture in Swiss cheese and the effects of moisture content upon quality and yield.

## METHODS

In the laboratory experiments, two cheeses were made daily from two lots of the same milk, under conditions and by methods as nearly as possible like those prevailing in the factories. The milk used in each kettle was weighed accurately in a receiving tank. Each cheese was weighed, and the percentage of moisture in the interior was determined, both when the cheese was one day old and when it was cut and graded. Both cheeses of each pair were made and cured in the same manner except for the experimental variation which was being studied. Plans were made to manufacture not less than 3 pairs of cheese under each experimental variation and, if the results were found to be inconsistent or if other important phases of the subject required more study, additional pairs were to be made so that averages for a larger number would be secured. Moisture data for green as well as cured laboratory cheese are presented, because it is believed that the former data are more representative of the effects of variables in the making process.

Laboratory cheeses were scored by an arbitrary, numerical scorecard system, which is intended to correspond as nearly as possible with the grading systems used by buyers and cheesemakers in the factories, and in which the relationship between grade and score is as follows: Fancy, 100 to 89; A or No. 1, 88 to 76; B or Special, 75 to 71; C or No. 2, 70 to 61; and D or Grinder, 60 or below. The principal points on the scorecard used, together

with the numerical value assigned to each, are as follows: eyes, 40; body and texture, 30; flavor, 20; and appearance, 10.

The data reported for factory cheese represent results obtained in the Bureau's portable laboratories, operating at factories in the States of Wisconsin and Ohio in cooperation with the Universities of these two States, and other results obtained in a factory in Pennsylvania. The study covers data on 226 cheeses made in 8 factories in Wisconsin, 160 cheeses made in 23 factories in Ohio, and 32 cheeses made in 1 factory in Pennsylvania—a total of 418 cheeses made in 32 factories. In the case of factory cheese the milk used in each cheese was not weighed and it was therefor not possible to obtain accurate figures for yield; neither was it feasible to obtain samples for analyses except at the time the cured cheeses were graded and sold.

## RESULTS

Statistical averages of data for 218 laboratory Swiss cheeses are presented in table 1, showing the relation of percentage of moisture in green cheese to

TABLE 1

*Data showing average score and yield of experimental Swiss cheese of different moisture content (218 60-lb. cheeses cured 2-1/2 to 3 months)*

| Moisture in green cheese | Number of cheeses | Average total score | Average eye score | Pounds cheese per cwt. milk | | Shrinkage in curing |
|---|---|---|---|---|---|---|
| | | | | Green | Cured | |
| *per cent* | | | | | | *per cent* |
| Above 39.7 | 15 | 65.4 | 16.8 | 8.98 | 8.14 | 9.35 |
| 39.1–39.7 | 27 | 74.8 | 24.0 | 8.80 | 7.98 | 9.32 |
| 38.5–39.1 | 63 | 74.6 | 23.7 | 8.85 | 8.04 | 9.15 |
| 37.9–38.5 | 80 | 74.0 | 22.8 | 8.82 | 8.04 | 8.84 |
| Below 37.9 | 33 | 73.4 | 22.3 | 8.89 | 8.12 | 8.66 |

total score, eye score, yield of cheese per hundred pounds of milk, and shrinkage of cheese in curing. Each cheese weighed between 56 and 61 pounds when made. Data in which the experiments involved abnormal variations are not included in the tabulations in table 1,—*i.e.,* cheese made from unclarified milk (table 3, variation No. 10), cheese made from milk which had been ripened with lactic starter (variation No. 6), and cheese made without the use of streptococcus starter (variation No. 1) are excluded.

The data indicate that a percentage of moisture above 39.7 in the green cheese is usually detrimental to the quality of the cheese. This detrimental effect was most evident in the eye formation. The defect known as "oversetting," a condition in which the eyes are too numerous and too small, was very pronounced in most of the high-moisture cheeses, and they usually tended to rise more rapidly and to a greater extent than low-moisture cheeses. Along with poor eye formation there often occurred, in high-moisture cheeses, a defective flavor usually characterized as "unclean."

Relationships between actual and theoretical yields, both for green and cured cheese, are shown in figure 1. The theoretical yields shown in line

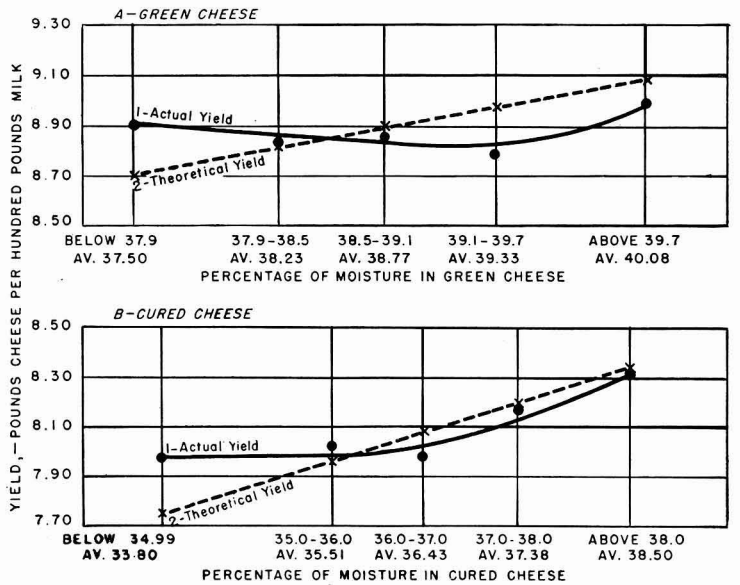


FIG. 1. Data showing average yield of experimental Swiss cheese of different moisture content (218 60-lb. cheeses).

2 are the yields that would be expected if the average yield of cheese in each moisture group was strictly proportional to the percentage of moisture. [The theoretical yield values were calculated by multiplying the average yield of cheese (green, 8.86 pounds; cured, 8.04 pounds) by the average percentage of dry matter (green, 61.45 per cent; cured, 63.81 per cent), and dividing the result by the percentage of dry matter in each respective moisture group.] A comparison of theoretical yields with actual yields shows that as the percentage of moisture in the cheese increased, the increase in yield was not proportional to the increase in percentage of moisture; as the percentage of moisture decreased, the actual yield was greater than the theoretical yield.

The data were therefore tabulated to determine the relationships between percentage of fat in kettle milk, percentage of moisture in cheese, and yield of cheese. The results shown in figure 2 indicate that as the percentage of fat in the kettle milk increased, the percentage of moisture in the green cheese tended to decrease. This fact has been pointed out previously by European workers (3, 9, 12, 16). It is indicated also in figure 2 that as the percentage of fat in the kettle milk increased, the yield of cheese tended to increase in spite of the decrease in moisture content.

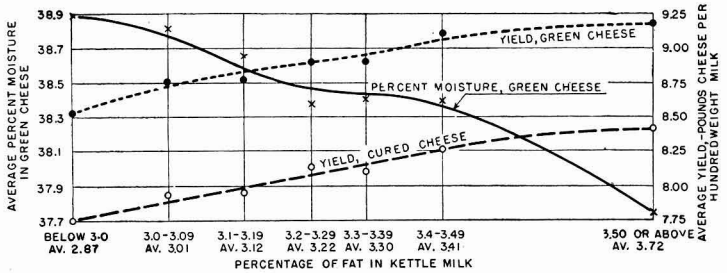


FIG. 2. Data showing average percentage of moisture and average yield of experimental Swiss cheese made from milk of different fat content (218 60-lb. cheeses).

Tabulation of data for 45 laboratory cheeses made from milks containing the same percentage of fat (3.1 per cent) indicated that, when the factor of fat variations in milk is eliminated, there is some increase in yield of cheese as moisture content increases, but the increase in yield is not proportional to the increase in moisture content. Cheeses in this group containing less than 35.0 per cent moisture when cured had an average cured yield of 7.92 pounds; those containing more than 38.0 per cent moisture had an average cured yield of 8.33 pounds.

While it is possible that the above results are not strictly typical of yields that may be expected to occur in factory cheese, for the reason that losses in moisture and in yield are smaller per unit of weight in the larger wheels, it is believed that the trends in the two cases are similar and that variations in the milk or making process will produce similar effects in the yield of green cheese in either case. Effects of manufacturing variations upon yield will be referred to below.

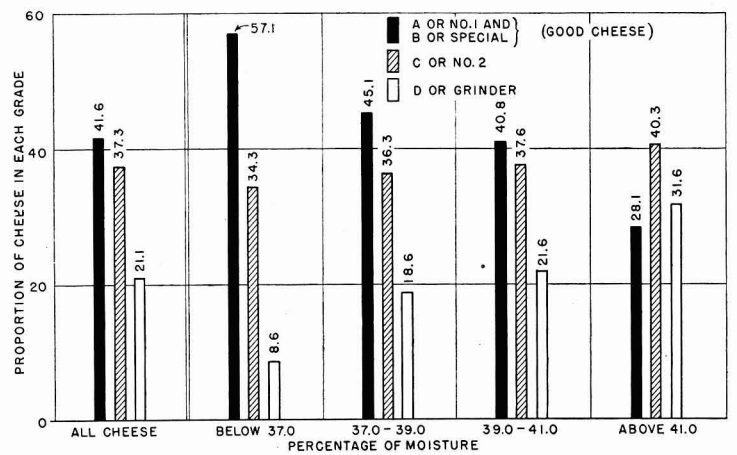


FIG. 3. Relation of moisture content to quality of Swiss cheese (418 factory cheeses cured approximately 2½ months).

Averages of data on moisture and quality for 418 factory cheeses are shown in figure 3. These data tend to confirm the laboratory results. The proportion of cheese of the A and B grades (good cheese) was relatively high in those cheeses which contained less than 37 per cent moisture—a figure which corresponds best with figures quoted above for foreign Swiss or Emmentaler cheese. Similarly, the proportion of cheese of the grinder grade (poor cheese) increased progressively as the average percentage of moisture increased from below 37 to above 41 per cent.

The average percentage of moisture in the 418 factory cheeses was 39.40. Averages of moisture data for the cheeses in each of the four grades were as follows: A, 55 cheeses, 38.70 per cent; B, 119 cheeses, 39.30 per cent; C, 156 cheeses, 39.46 per cent; and D, 88 cheeses, 39.88 per cent. These figures show a consistent downward trend in quality as moisture content increased.

Average moisture figures and grades were also tabulated for each factory. These were divided into two groups, as follows: (a) 8 factories in each of which the average percentage of moisture in the cheeses sampled was greater than 39.4 per cent, and (b) 24 factories in each of which the average percentage of moisture in the cheeses sampled was less than 39.4 per cent. Of 226 cheeses sampled in the first group (moisture above average), 31.0 per cent were graded No. 1 or special, corresponding to A or B grade (good cheese); 39.4 per cent were graded No. 2, corresponding to C grade; and 29.6 per cent were graded grinder, corresponding to D grade. Of 192 cheeses sampled in the second group (moisture below average), 54.2 per cent were graded No. 1 or special (good cheese); 34.9 per cent were graded No. 2; and 10.9 per cent were graded grinder. The greater proportion of good cheese was produced in those factories in which cheese of relatively low moisture content was manufactured.

The possibility was considered as to whether the effect of poor quality of milk might have predominated in producing poor average quality in high-moisture cheese. Tabulations of the data for factory cheese showed, however, that the average methylene blue reduction time of the milks used in the cheeses in the low-moisture groups shown in figure 3 was actually slightly shorter than that of the milks used in cheeses in the high-moisture groups. The data indicated that the use of milk having a short methylene blue reduction time resulted in a general but slight tendency toward a decrease in moisture content in the cheese; this is probably the result of the curd-drying effect of overripe milk, resulting from a rapid production of acidity in the kettle and on the press when such milk was used. However, it has been shown by Rogers, Hardell and Feutz (10), and also by Erekson and his coworkers (5), that a short reduction time usually results in a rather markedly detrimental effect upon the average quality of cheese. They found that the proportion of good cheese was considerably greater when the reduction time was more than 3 hours than when it was less.

The results for factory cheese were tabulated on the basis of a combination of methylene blue reduction time and moisture content of cheese. Resulting data are illustrated in figure 4. It is shown that a methylene

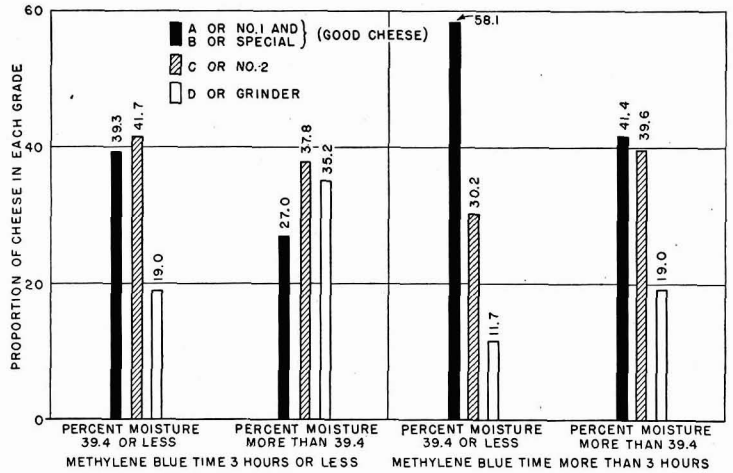


FIG. 4. Relation of methylene blue reduction time of milk and percentage of moisture in cheese to quality of Swiss cheese (418 factory cheeses).

blue reduction time of more than 3 hours together with a relatively low percentage of moisture produced the greatest proportion of good cheese, while a reduction time of less than 3 hours together with a relatively high percentage of moisture in the cheese produced the greatest proportion of grinders or cheese of poor quality.

Our data did not show that the factors that cause an increase in moisture content always result in impairment of quality of cheese. They did indicate that the chances of making a relatively large proportion of good cheese are impaired when the moisture content is excessively high. Other factors, in addition to those considered in this paper, unquestionably play a part in influencing quality.

It will be noted that the range of values for moisture in laboratory green cheese shown in table 1 is lower than the range in factory cured cheese in figure 3. We have found it difficult, when using normal methods, to incorporate as large a percentage of moisture in laboratory cheese as is often present in factory cheese. Analyses show that one reason for this difference is the fact that smaller cheeses lose moisture somewhat more rapidly than larger ones by drainage on the press. A larger proportion of the milk used in certain cheese-producing areas has a relatively lower fat and total solids content and a softer curd—factors which tend to produce an increase in moisture content. Moreover, as has been demonstrated (13) in the case of the

Cheddar variety, smaller cheeses lose a greater percentage of moisture than larger ones by evaporation during curing.

Before results of variations in the making process are discussed, it seems desirable to present data showing temperature conditions and the amount and rate of moisture loss from the curd during this process. Such data, taken from work on Emmentaler cheese reported by Koestler (7) and also from our results on laboratory Swiss cheese, are shown in table 2. The lab-

TABLE 2

*Data showing temperature conditions and losses of moisture during Swiss cheese manufacturing process*

| Stage in manufacturing process | Data by Koestler, average of 4 cheeses | | | Data by this laboratory, average of 30 cheeses | | | |
|---|---|---|---|---|---|---|---|
| | Temp. | Moisture | Loss of moisture | Time elapsed | Temp. | Moisture | Loss of moisture |
| | °C. | % | % | min. | °C. | % | % |
| Before curdling | 31.75 | 88.4 | 0.0 | 0 | 32.5 | 87.8 | 0.0 |
| Beginning of heating | 30.75 | 63.3 | 25.1 | 77 | 32.0 | 64.9 | 22.9 |
| End of heating | 53.0 | 54.8 | 8.5 | 31 | 53.0 | 53.6 | 11.3 |
| Dipping | 50.3 | 51.8 | 3.0 | 48 | 50.5 | 52.6 | 1.0 |
| 3 hrs. after dipping | ..... | ..... | ..... | ..... | 48.3 | 42.1 | 10.5 |
| 21 hrs. after dipping | ..... | 37.0 | 14.8 | ..... | 39.0 | 39.1 | 3.0 |

oratory data show percentages of moisture in the uncoagulated kettle milks, in samples of curd taken from the kettle with a strainer and allowed to drain for 2 minutes before being analyzed, and in plug samples taken from the interiors of the cheeses. It is shown that the greatest losses of moisture from the curd occur during the time when rennet action is most rapid after curdling, and again when the curd is dipped and placed on the press. The greatest proportion of whey loss from the cheese after dipping occurs very early on the press, at a time when the activity of the streptococci is relatively great in comparison with that of the lactobacilli.

There is presented in table 3 a list of those factors which in our experiments were found to influence the percentage of moisture in experimental Swiss cheese and the resulting average yields. Only a few of the apparently more important effects of the variations shown will be discussed.

In variation No. 1, all cheeses made without streptococcus starters were grinders, and their yields and moisture contents were comparatively high.

Results shown in variation No. 3 confirm the work of Koestler (7), who found that fine harping caused a considerable increase in moisture content. Even though fine particles contained a smaller percentage of moisture (and fat) than large ones, cheese moisture content was greater because of more retention of moisture on the greatly increased surface area of fine particles, and because fine particles tend to cause a stopping-up or clogging of drainage capillaries. In our experiments, the finely-harped cheeses were not in-

TABLE 3

*Data showing effects of variations in the making process upon the average moisture content and yield of experimental Swiss cheese**

| Variation in making process | Number of pairs | Moisture in green cheese | | Yield cured cheese per cwt. milk | |
|---|---|---|---|---|---|
| | | Ave. | Diff. | Ave. | Diff. |
| | | % | % | lb. | lb. |
| 1. No streptococcus starter | 3 | 40.16 | | 8.39 | |
| 25 cc. streptococcus starter† | | 38.46 | −1.70 | 8.16 | −0.23 |
| 2. Holstein milk standardized to 3.5% fat | 3 | 38.80 | | 7.94 | |
| Jersey milk standardized to 3.5% fat | | 37.50 | −1.30 | 8.25 | +0.31 |
| 3. Harped fine | 5 | 38.97 | | 7.85 | |
| Harped coarse | | 37.94 | −1.03 | 7.96 | +0.11 |
| 4. 10% water added to kettle milk | 5 | 39.49 | | 7.74 | |
| No water added | | 38.47 | −1.02 | 7.95 | +0.21 |
| 5. Normal milk standardized to 2.9% fat | 30 | 38.68 | | 7.68 | |
| Normal milk standardized to 3.4% fat | | 37.69 | −0.99 | 8.23 | +0.55 |
| 6. Milk not ripened | 5 | 38.40 | | ..... | |
| Milk ripened with lactic starter | | 37.50 | −0.90 | ..... | ..... |
| 7. 0.03% sodium citrate added to milk | 5 | 40.08 | | 7.78 | |
| No citrate added | | 39.22 | −0.86 | 7.92 | +0.14 |
| 8. Heated in 26 minutes | 10 | 38.73 | | 8.14 | |
| Heated in 60 minutes | | 37.91 | −0.82 | 8.03 | −0.11 |
| 9. 9 cc. streptococcus starter† | 4 | 39.37 | | 7.91 | |
| 27 cc. streptococcus starter† | | 38.87 | −0.50 | 7.85 | −0.06 |
| 10. Milk not clarified | 18 | 38.70 | | ..... | |
| Milk clarified | | 38.20 | −0.50 | ..... | ..... |
| 11. 5 cc. rennet† | 3 | 38.43 | | 8.14 | |
| 10 cc. rennet† | | 38.00 | −0.43 | 8.12 | −0.02 |
| 12. Set at 31° C. | 3 | 38.63 | | 7.70 | |
| Set at 35° C. | | 38.20 | −0.43 | 7.66 | −0.04 |
| 13. 20 cc. lactobacillus starter } † 12.5 cc. streptococcus starter } | 6 | 38.87 | | 8.15 | |
| 60 cc. lactobacillus starter } † 40 cc. streptococcus starter } | | 38.49 | −0.38 | 8.19 | +0.04 |
| 14. Cooked 50.5° C.; stirred 16 min. | 6 | 38.61 | | 8.25 | |
| Cooked 54° C.; stirred 60 min. | | 38.24 | −0.37 | 8.12 | −0.13 |
| 15. 5% cold water added before dipping | 4 | 38.90 | | 8.20 | |
| No water added | | 38.65 | −0.35 | 8.29 | +0.09 |
| 16. Cut 28 min. after setting | 3 | 38.93 | | 7.96 | |
| Cut 42 min. after setting | | 38.59 | −0.34 | 7.90 | −0.06 |
| 17. Combination of above variations‡ | | | | | |
| Wet | 6 | 40.50 | | 8.47 | |
| Dry | | 37.53 | −2.97 | 8.43 | −0.04 |

* The following variations resulted in average difference of less than 0.25 per cent moisture:

Foreworking 60 min. compared with 20 min.; yield 8.13 compared with 8.25.
Cooking to 54.5° C. compared with 50.5° C.; yield 8.05 compared with 8.07.
Stirring out 50 min. compared with 15 min.; yield same.
Heavy pressing compared with light pressing; yield 8.11 compared with 8.08.

† Per cwt. milk.

‡ Conditions combined included variations Nos. 3, 8, 9, 11, 12, and 14.

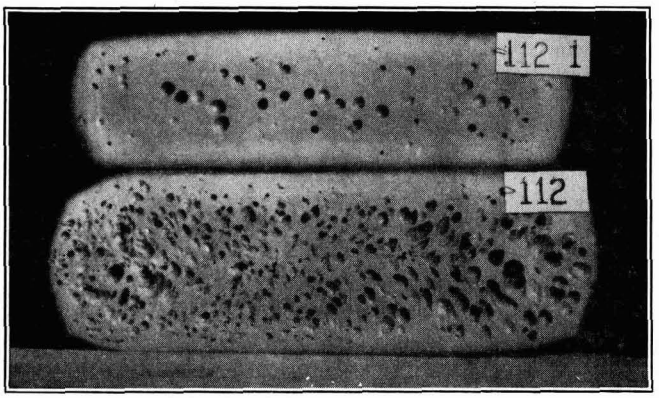


FIG. 5. Low-moisture and high-moisture cheeses made from separate portions of the same milk: 112—1, 37.51% moisture (green); score, 74 (cured)—112, 40.55% moisture (green); score, 50 (cured).

ferior in quality. The yield of cheese, however, was reduced 0.11 pound by fine harping. The wheys from the 5 finely-harped cheeses contained an average of 0.64 per cent fat and 6.80 per cent total solids; corresponding figures for the coarsely-harped ones were 0.52 and 6.65 per cent.

Effects of variations in fat content of milk, referred to earlier in this paper, are pointed out in variation No. 5. Milk standardized to an average of 2.9 per cent fat yielded green cheese containing an average of 38.68 per cent moisture and 44.4 per cent fat in dry matter, and portions of the same milk standardized to 3.4 per cent fat yielded cheese containing an average of 37.69 per cent moisture and 49.1 per cent fat in dry matter. The high-fat cheeses, which contained the least moisture, were generally superior in quality, especially with respect to texture and flavor. The average yield of cured cheese of the low-fat group was 7.68 pounds per hundred pounds of milk and that of the high-fat group was 8.23 pounds. Corresponding yields of cured cheese per pound of fat in milk were 2.66 and 2.34 pounds, respectively. Methods of standardization, and effects of percentage of fat on quality, will be discussed in a later paper.

The data shown under variations No. 8, 14, and 16 indicate that expulsion of moisture is favored by extending the time of certain stages in the making process. Our results indicate that this is particularly true when acidity is developing rapidly in the kettle contents. Under this condition the drying effect of a slow, prolonged heating period is especially marked. Vas (14) has shown that long-continued heating not only increases the specific gravity and decreases the water-holding capacity and surface adhesiveness of the granules, but also stiffens the granular structure and promotes the whey-carrying effectiveness of the capillaries through which drainage occurs after dipping.

Long foreworking resulted in a slight decrease in yield, but had little effect in decreasing moisture content except when acid was being formed rapidly in the kettle.

In variations Nos. 1, 9, and 13, pH values at dipping and at 3 hours later showed relatively rapid development of acidity in the cheeses containing the larger amounts of starter; the relatively lower moisture content in these cheeses demonstrates the effect of acidity in drying the curd. Numerous other experiments have demonstrated conclusively that acid development is an important factor in drying the cheese in the kettle and on the press. The expulsion of moisture in the presence of acid is readily explained by the fact that, as the reaction of the curd changes toward pH 4.6, which is the isoelectric point and the point of least solubility of casein, the curd shrinks and tends to lose its ability to combine with or hold water. It has been shown (2), however, that when acidity develops rapidly near the rind and slowly in the interior, the high-acid rind forms a barrier that hinders proper drainage of the cheese on the press. Such a condition may exist when the number or activity of streptococcus starter organisms, which grow relatively early in the interior of the cheese, is low in comparison with that of the lactobacilli. Our results indicate that an increase in the amount or activity of the streptococcus starter has a somewhat greater effect in promoting drainage than an increase in the amount or activity of the lactobaccillus starter.

In variation No. 6, cheese made from milk which had previously been ripened slightly with a lactic starter containing only *Streptococcus lactis* organisms showed a relatively rapid development of acidity on the press after dipping, even though the activity of these organisms has been shown to be stopped by the temperature used in the cooking process (6). These ripened-milk cheeses in which acid was produced rapidly contained some glass (glaesler defect) and were slightly short and inelastic in texture and inferior in quality.

Dorner and Stähli (4) found that when water was added to the kettle contents just previous to dipping (variation No. 4), the resulting dilution of the whey caused a decrease in the amount of lactose contained in the cheese and hence a decrease in the ultimate amount of acid which was formed in the one-day-old cheese. The higher pH value (lower acidity) of the one-day-old cheese resulted in an increase in eye formation. These observations are confirmed by our results—the addition of water caused an average decrease in final acidity (increase in pH value) in the one-day-old cheese, and the tendency toward oversetting was greatest in those cheeses having the highest average pH values when one day old.

Orla-Jensen (9) found that increasing the cooking temperature from 48° to 56° C. caused the percentage of moisture in the cured cheese to decrease from 36.64 to 35.33 per cent, and he quoted earlier work of Schaffer which

showed a similar drying effect resulting from high cooking. Both of these workers found that the use of high cooking temperatures caused a decrease in the rate of protein decomposition and ripening in the cheese. Orla-Jensen believed that the cooking temperature should be high enough to aid in drying the cheese curd sufficiently without the necessity of a long stirring-out period, but not sufficiently high to seriously inhibit ripening and the formation of eyes. He believed that the use of a relatively high cooking temperature together with an addition of eye-forming organisms would increase the possibility of making cheese of relatively high quality. Our laboratory results, in general, confirm the latter observation.

In our experiments, an increase of 3° C. in cooking temperature caused an average decrease of less than 0.25 per cent in moisture in the green cheese. Similarly, the decrease was very small when the duration of the stirring-out period was increased markedly. A combination of high cooking temperature with long stirring-out time (variation No. 14), not ordinarily used in factory practice, resulted in a decrease of 0.37 per cent moisture, and resulted in a slight improvement in the average quality of the experimental cheeses.

High cooking temperatures regularly caused the curd to be relatively dry at the end of the cooking and of the stirring-out period. However, high cooking temperatures retarded acid formation on the press rather markedly, and the high-cooked cheeses lost moisture comparatively slowly after dipping, presumably because of slow pH change. When the kettle contents were stirred out for relatively long periods, it was found that cooling was more rapid in the kettle than on the press. Long stirring-out periods resulted in low dipping temperatures which served to accelerate, to a slight extent, both acid formation and drainage on the press.

Analytical results showed that the effects of the variations listed in table 3 were not strictly cumulative. In securing the data shown in variation No. 17, six pairs of cheese were made in which the following factors were combined to produce high moisture content in one cheese of each pair: less rennet, less streptococcus starter, lower setting temperature, finer harping, lower cooking temperature, and a shortening of each stage of the making process; in addition, in two cheeses out of the six, the percentage of fat in the kettle milk was reduced to 2.9 as compared with 3.5. By combining these factors against an opposite condition in each case, it was found that the percentage of moisture could be varied within a range of about 3 per cent. This range evidently would differ with conditions involving principally the properties of the milk and the size of the cheese.

In figure 5 is shown a photograph of a pair of laboratory cheeses in which the lower cheese contained a relatively high percentage of moisture and shows oversetting and irregular eye formation. These cheeses were made in experiments described under variation No. 17, table 3.

It has been pointed out above that a relatively low percentage of fat in kettle milk resulted in a relatively high percentage of moisture but a low yield in cheese, and that fine harping had a similar effect. Other variations in which relatively high moisture content was accompanied by a decrease in yield were: No. 2, the use of Holstein or low-solids milk; No. 4, the addition of water to milk; No. 7, the addition of sodium citrate, which produced soft-curd milk; No. 13, the use of relatively small amounts of starters; and No. 15, the addition of water before dipping.

Studies of the analytical and yield data obtained in the present work indicated that, in addition to the physical effect of the amount of moisture present, the following factors tend to influence yield:

1. The use of milk standardized to a relatively low fat content results in relatively high moisture content in the cheese but decreases the yield because of the decrease in amount of fat in the cheese.

2. Milk low in solids-not-fat content tends to have a low curd tension; the use of such milk results in relatively high moisture content in cheese but decreases the yield because of low solids content and because of an increase in the proportion of solids lost in the whey.

3. High-moisture cheese loses a relatively large proportion of its moisture, and of its yield, during curing.

4. Some of the procedures used to increase moisture content cause losses in yield by causing an increased loss of solids in the whey.

## SUMMARY

Correlations of analytical data with grades of 218 experimental and 418 factory Swiss cheeses indicated that the presence of an excessive amount of moisture is generally detrimental to the quality of the cheese.

Laboratory results on yields of cheese per hundred pounds of milk indicated that some of the manufacturing variations which may be used to bring about the inclusion of excess moisture actually result in decreases in yield, and that in general the inclusion of a comparatively large amount of moisture does not result in sufficient increase in yield to justify the practice.

A study is presented showing effects of numerous variables in the milk and making process upon the moisture content of the cheese.

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# A STUDY OF THE CHARACTERISTICS OF A MILK SUPPLY AS RELATED TO THE MANUFACTURE OF PLAIN CONDENSED SKIMMILK FOR ICE CREAM MAKING[1]

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It is often economically desirable for dairy manufacturing plants to be self-sufficient in supplying the milk ingredients of their products. In the manufacturing of ice cream a concentrated milk is used as a source of added serum solids to improve the body and texture. Since the production of milk fluctuates during the seasons it would be desirable, perhaps, to concentrate the solids of the surplus milk to be used during the period of reduced production.

It was in an effort to store these milk solids with a minimum of processing that led to the freezing and storing of condensed skimmilk by some ice cream manufacturers. In many instances this practice of condensing the surplus skimmilk, freezing and holding it frozen until used to make the ice cream mix, has been carried out successfully; yet in other cases it has proved to be unsatisfactory because after the skimmilk had thawed it was found that precipitation of the protein had occurred.

Therefore, if it were possible to determine by some chemical or physical test the degree of stability of the protein fraction of the milk supply, the suitability of the milk to being stored frozen in the form of condensed skimmilk could be determined.

## STATEMENT OF PROBLEM

The purpose of the investigation was to study the characteristics of a milk supply which appears to be associated with the ability of the protein fraction to remain stable after the skimmilk obtained by centrifugal separation had been condensed and then stored frozen. The study was conducted by making chemical and physical tests on representative samples of milk taken at specific intervals in the manufacture and storage of the plain condensed skimmilk. Physical and chemical tests were also used to determine the effect of a change in the characteristics of condensed skimmilk during storage upon an ice cream mix and the resultant ice cream.

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[2] The data presented in this paper are from a study made by the junior author under the supervision of the senior author in partial fulfillment of the work required for the degree of Master of Science.

## REVIEW OF LITERATURE

Reichart and Corley (8) have presented a review of the work done previous to 1938. In their work they found that after storing plain condensed skimmilk at −17.8° C. (0° F.) for three months, the condensed skimmilk was coarser than when first made, and at the fourth month a partial gel had been formed. From the fifth month on the milk had formed a complete gel and in addition, at the eighth and ninth months some wheying off was observed on thawing. However, they found that in spite of the poor appearance of the frozen condensed skimmilk after being melted, the appearance of the final ice cream mix was not affected and they had no trouble in processing it. They recommended, however, that the skimmilk should not be stored over six months.

Openlander and Erb (6) reported that condensed skimmilk if properly frozen and stored could be used as a satisfactory source of serum solids. They used a storage period of 13 weeks and found that the first noticeable defect which might occur in the ice cream made from frozen condensed skimmilk was the curdled appearance on melting.

Winn (13) found that plain condensed skimmilk, as well as sweetened condensed skimmilk, could be frozen and stored at −17.8° C. (0° F.) for periods up to 3 months without any detrimental effect upon their physical appearance. He found, however, that superheated condensed skimmilk showed signs of protein precipitation at the end of one month of storage, and the precipitation increased rapidly as the storage period continued up to 3 months.

## EXPERIMENTAL METHODS

The whole milk supply used in this study came from an area adjoining Lincoln, Nebraska. After being examined to make sure it had no objectionable off-flavor, the milk was pasteurized at 145° F. for 30 minutes; then cooled to a temperature of 90–100° F. before being separated. Immediately after separation the skimmilk was preheated in 150-gallon lots to 150° F. It was then drawn into a Roger 26-inch vacuum pan (stainless steel) where it was condensed under a vacuum of about 25 inches, until the total solids content as determined by the Baume hydrometer was approximately 33.5 per cent.

Between 50 and 52 pounds of the condensed skimmilk were placed in new 5-gallon tinned lard cans and stored at −17.8° C. (0° F.). Altogether, 9 lots of whole milk were processed at approximate intervals of 4 weeks from October through May.

A 300-pound ice cream mix was made from each lot of condensed skimmilk when fresh and again after being stored frozen for four weeks. The frozen condensed skimmilk was held in water at 21.1° C. (70° F.) over night to allow it to melt before it was incorporated in the ice cream mix. The mix

was calculated to contain 14 per cent milk fat, 10 per cent serum solids, 15 per cent sugar, and 0.25 per cent gelatin. In each case the cream and skimmilk required to furnish the milk fat and serum solids not furnished by the condensed skimmilk was not more than 24 hours of age. After being held at 71.1° C. (160° F.) for 20 minutes the mix was homogenized with a two-stage homogenizer (Manton-Gaulin) at 3000-pound pressure with 500-pound on the second stage.

After aging 24 hours at 5 to 6° C. (41 to 43° F.) the ice cream mix was frozen in a horizontal, direct-expansion 40-quart freezer. Three batches of mix were frozen, the first one being a preliminary batch. The amount of mix used in the preliminary trial was 45 pounds, while in the following two runs the amount was 42 pounds. The temperature of the ice cream mix at the beginning of the freezing process, the time required to lower the temperature of the contents of the freezer to – 4.4° C. (24° F.) and the temperature of the refrigerant when this temperature of – 4.4° C. (24° F.) was reached were recorded.

Overrun readings were taken by means of the Mojonnier overrun tester immediately after the refrigerant was shut off and at minute intervals thereafter until the maximum overrun had been obtained.

*Proceedings of sampling.* Quart samples of the whole milk were obtained before and after pasteurization of the skimmilk before preheating, and of the condensed skimmilk while fresh and after being stored frozen 4 weeks. A sample of each ice cream mix was taken at the time of freezing and a sample of ice cream was taken in quart sealright containers directly from the freezer at 100 per cent overrun and held in the hardening room at – 17.8° C. (0° F.). The other samples were held in an ice and water bath.

*Analytical procedure.* The raw whole milk was analyzed to determine whether or not it was normal in chemical composition. Milk fat and total solids were determined by the Mojonnier method (4). The lactose, total protein, casein, and ash were determined according to the methods outlined in A.O.A.C. (5). A modification of the method of Rosswell (10) was used to determine the chloride content. Forty ml. of distilled water were added to 10 grams of milk in an Erlenmeyer flask and, after thorough mixing, the contents were titrated with $AgNo_3$ of such a concentration that 1 ml. equalled 1 milligram of chlorine in 10 grams of the sample. One ml. of a 10 per cent solution of potassium chromate was added to the flask as an indicator.

The pH of the samples of whole milk and skimmilk was determined by means of the quinhydrone electrode using a type K Leeds and Northrup potentiometer at 25° C. (77° F).

The titratable acidity was determined by titrating 25 grams of the sample which had been diluted with 25 ml. of $CO_2$ free double distilled $H_2O$, with 0.1 normal NaOH using 10 drops of a 1 per cent solution of phenolphthalein.

The alcohol test of Dahle and Peyenson (1) was used to indicate protein stability and the least amount of 95 per cent ethyl alcohol required to bring about the first noticeable indication of coagulation in a 5 ml. sample was designated as the alcohol number. In all cases water was added prior to the alcohol so that the total volume of the addition (water plus alcohol) amounted to 10 ml. Also, the phosphate test of Ramsdell, Johnson and Evans (7) was used. However, when applied to condensed skimmilk the number of seconds submersion in the boiling water required to bring about indications of coagulation were recorded.

The milk fat and total solid content of the ice cream mixes were determined in duplicate by the Mojonnier method. The titratable acidity and pH were determined as they were in the case of the samples of milk. A heat coagulation test based on a modification of the method of Howat and Wright (3) was used. Three ml. of the ice cream mix were pipetted into a $15 \times 125$ mm. Pyrex test tube which was then closed with a rubber stopper. The stoppered test tube was then immersed in an oil bath maintained at 110° C. $\pm$ 0.5° C. (230° F.) until visible coagulation occurred; the time in minutes required to bring this about was recorded. The viscosity of the ice cream mix was determined by means of a Gramercy Model MacMichael Viscosimeter operated at 20 r.p.m. using a No. 30 wire and a 100 ml. sample at 5° C. (41° F.).

Approximately 10 days after freezing, the quart ice cream samples were removed from the hardening room and cut into two equal portions of one pint each. One pint was scored for body and flavor. The second pint was set upon a $\frac{1}{4}$-inch mesh wire, resting upon the rim of a heavy 5-inch funnel the end of which led into a mouth of a 100 ml. graduated cylinder which had been previously tared. The number of minutes required for the first drop, first 50 ml. and the first 100 ml. of melt to collect were recorded as was the weight of the first 100 ml. of the melt. The sample of ice cream was allowed to melt at room temperature, in order to simulate actual practical conditions as much as possible.

## EXPERIMENTAL RESULTS

*Effect of processing upon the pH, titratable acidity, alcohol number and phosphate test.* Chemical analysis showed that all nine lots of milk used were normal in regard to the amount present of each of the constituents determined. The pH of the raw whole milk did not vary significantly from the usual range of 6.4 to 6.8.

As shown in table 1 it was found that pasteurization increased the pH, decreased the titratable acidity and tended to increase the alcohol number. The titratable acidity values of the whole milk ranged from .150 to .177 per cent calculated as lactic acid. It was found that the titratable acidity of the raw whole milk decreased from October through February, then from March through May it tended to increase again. The pH of the skimmilk

TABLE 1

*Effect of pasteurization on the pH, titratable acidity and alcohol number of whole milk*

| Date processed | Tests used | | | | | |
|---|---|---|---|---|---|---|
| | Before pasteurization | | | After pasteurization | | |
| | pH | Titratable acidity | Alcohol number | pH | Titratable acidity | Alcohol number |
| 10–21–38 ..... | 6.58 | .18 | 5.0 | 6.60 | .17 | 6.0 |
| 11–18–38 ..... | 6.59 | .17 | 6.5 | 6.61 | .16 | 7.0 |
| 12–28–38 ..... | 6.59 | .16 | 8.0 | 6.63 | .14 | 8.0 |
| 1–27–39 ..... | 6.54 | .16 | 7.0 | 6.57 | .15 | 8.0 |
| 2–24–39 ..... | 6.68 | .15 | 8.5 | 6.70 | .15 | 8.5 |
| 3–24–39 ..... | 6.70 | .17 | 8.5 | 6.71 | .16 | 8.5 |
| 4–14–39 ..... | 6.73 | .16 | 8.5 | 6.70 | .15 | 9.0 |
| 5– 5–39 ..... | 6.50 | .17 | 8.0 | 6.60 | .17 | 8.2 |
| 5–26–39 ..... | 6.37 | .17 | 8.3 | 6.48 | .16 | 8.5 |

was decreased noticeably by condensing. Before condensing, the range in pH for the skimmilk was from 6.64 to 6.69; after condensing, the range was from 6.04 to 6.33. There was no apparent seasonal trend in the pH of either product. The titratable acidity of the skimmilk was greatly increased by condensing. The increase was proportional to the increase in solids content. All samples were normal and there was some indication that the per cent acidity found in the fresh skimmilk followed the same trend as it did in the raw whole milk.

The skimmilk had a higher alcohol number before condensing than the original milk supply, but a lower alcohol number after being condensed due to the influence of the total solids of the products. The range in alcohol numbers was not great, being from 8.5 to 9.5 for the skimmilk and 4.0 to 5.0 for the condensed skimmilk but with no definite seasonal trend shown.

In the case of the phosphate test it was found that (with one exception) the same fluctuations of protein stability were found in the condensed skimmilk as was indicated by the alcohol number. This one exception was in the lot of condensed skimmilk processed April 14, 1939. In this instance the protein was the most stable of any lot of condensed skimmilk studied, as was shown by the phosphate coagulation time of 70 seconds, yet this increased stability over the average of 55 seconds was not shown by the alcohol number of 4.0 which was lower than some of the other lots of condensed skimmilk prepared in this study.

*Effect of freezing and storing upon the character of the condensed skimmilk.* No evidence of wheying off could be seen at any time in the frozen condensed skimmilk after being stored at −17.8° C. (0° F.) for 4 weeks. Other than being slightly sandy, the frozen condensed skimmilk melted down to yield a product similar to the fresh condensed skimmilk in body and texture.

Freezing and storing of the condensed skimmilk for 4 weeks caused an increase in titratable acidity and with one exception a decrease in the pH.

With one exception the protein stability of the condensed skimmilk as measured by the alcohol test was affected only slightly by storing frozen for 4 weeks. The data found are shown in table 2. In the remaining 8 trials the

TABLE 2

*The effect of storing plain condensed skimmilk at −17.8° C. (0° F.) on certain properties*

| Date condensed | Total solids | Length of storage period | Test used | | | |
|---|---|---|---|---|---|---|
| | | | pH | Titratable acidity | Alcohol number | Phosphate test coagulation time |
| | % | *weeks* | | % | | *seconds* |
| October 21, 1938 | 33.97 | 0 | 6.148 | .833 | 4.5 | 50 |
| | 33.97 | 4 | 5.978 | .867 | 2.5 | 50 |
| November 18, 1938 | 35.20 | 0 | 6.203 | .732 | 4.5 | 50 |
| | 35.20 | 4 | 6.086 | .754 | 4.5 | 50 |
| December 28, 1938 | 35.58 | 0 | 6.104 | .418 | 5.0 | 60 |
| | 35.58 | 4 | 6.071 | .572 | 4.5 | 50 |
| January 27, 1939 | 33.20 | 0 | 6.038 | .637 | 4.5 | 55 |
| | 33.20 | 4 | 6.198 | .675 | 4.5 | 50 |
| February 27, 1939 | 32.95 | 0 | 6.332 | .641 | 4.0 | 55 |
| | 32.95 | 4 | 6.285 | .673 | 3.8 | 50 |
| March 24, 1939 | 32.13 | 0 | 6.278 | .583 | 4.5 | 60 |
| | 32.13 | 4 | 6.218 | .677 | 4.5 | 60 |
| April 14, 1939 | 31.58 | 0 | 6.158 | .640 | 4.0 | 70 |
| | 31.58 | 4 | 6.107 | .678 | 3.8 | 60 |
| May 5, 1939 | 31.80 | 0 | 6.159 | .662 | 4.0 | 55 |
| | 31.80 | 4 | 6.139 | .681 | 4.0 | 55 |
| May 26, 1939 | 33.76 | 0 | 6.140 | .704 | 4.5 | 55 |
| | 33.76 | 4 | 6.129 | .715 | 4.3 | 50 |

alcohol number was slightly lower in 50 per cent of the trials. The season of the year apparently had no great influence upon the stability of the protein since the slight decrease which occurred was found in alternate months during this study.

The stability of the protein fraction of the condensed skimmilk as determined by the phosphate test was found to have decreased after being held frozen for 4 weeks in 5 of the 9 lots of condensed milk studied. In attempting to correlate the alcohol and phosphate tests, it was found that in 4 of the 9 lots of condensed skimmilk the stability of the protein fraction was found to have decreased by both the alcohol and phosphate tests. In 3 lots the stability as measured by these two tests remained the same, and in the remaining 2 lots of condensed skimmilk the results were inconsistent.

*Comparison of the pH and titratable acidity of the ice cream mixes made from fresh or stored frozen condensed skimmilk.* The pH of the 18 mixes made fell within the range of 6.1 to 6.4 which according to Sommers (11) is the range in pH for normal ice cream mixes. The pH of the ice cream mix containing the condensed skimmilk which had been stored frozen was lower

than that of the ice cream mix made from the same condensed skimmilk while fresh in 5 of the 9 comparisons, while the pH of the condensed skimmilk itself was lowered in 8 of the 9 lots. It was also found that while the titratable acidity of the condensed skimmilk increased after storage, the titratable acidity of the mix was increased in only 2 of the 9 trials by using condensed skimmilk which had been stored frozen 4 weeks at −17.8° C. (0° F.).

*Comparison of the viscosity and resistance to heat coagulation of ice cream mixes made from fresh or stored frozen plain condensed skimmilk.* From the data obtained in determining the viscosity of the ice cream mixes it was evident that the difference in viscosity in the mixes containing fresh condensed skimmilk as compared to the mixes made from the stored frozen condensed skimmilk showed no definite consistent trend due to storing.

The results indicated that the use of the condensed skimmilk which had been frozen had no definite influence upon the resistance to coagulation by heat of the resulting ice cream mixes when the time required to bring about coagulation at 110° C. (230° F.) was determined.

*The effect of using fresh or stored frozen condensed skimmilk on the whipping ability of the ice cream mix.* The average whipping ability of the 9 pairs of mixes studied is shown in figure 1. In 6 of the 9 comparative pairs the mix containing the stored frozen condensed skimmilk as a source of added serum solids whipped slower and did not attain as great a maxi-

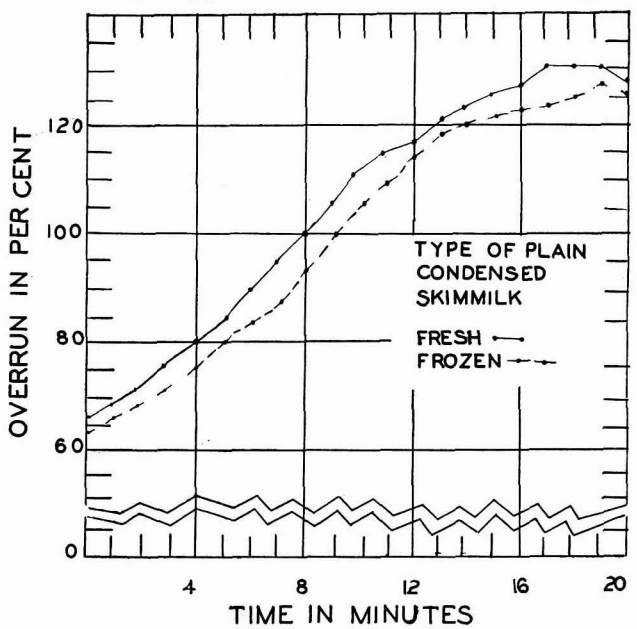


FIG. 1. Whipping ability of ice cream mixes containing fresh condensed skimmilk compared to mixes containing condensed skimmilk stored at 0° F. for four weeks.

mum overrun as the mix containing the same condensed skimmilk while fresh. It was interesting to note that at two different times when two mixes were prepared containing the same ingredient other than the condensed skimmilk which was prepared 4 weeks apart, that the whipping properties were the same even though one mix contained fresh condensed skimmilk and the other mix the condensed skimmilk prepared 4 weeks previous and held frozen until used. This would seem to indicate that any difference in the whipping properties of ice cream mixes containing fresh or frozen condensed skimmilk is materially affected by the influence of other ingredients used.

*Effect of using fresh or stored frozen plain condensed skimmilk on the quality of the ice cream.* The ice cream samples were scored after having been in the hardening room for 10 to 14 days. The difference in flavor scores was not significant. Of the criticisms given, four of the samples were criticized for having a slight condensed milk flavor, and in each case frozen condensed skimmilk had been used. The intensity of this flavor was not sufficient to justify the statement that frozen condensed skimmilk should not be used. Seven of the 18 ice creams were criticized for having a slight "cooked" flavor probably due in part to pasteurizing at 160° F. for 20 minutes. In the ice creams made during April and May a feed flavor was very noticeable due to the type of pasture to which the producing animals had access.

In regard to texture, one ice cream was criticized for being "icy." The other 17 were criticized for being either "slightly coarse" or "slightly icy." Our results showed that there was no significant raising or lowering of the score for either body and texture or flavor due to the use of stored frozen plain condensed skimmilk rather than the fresh product.

*Effect of using stored frozen plain condensed skimmilk upon the melting characteristics of the finished ice cream.* Widely varying results were obtained from the melt down tests. The reason for these results might have been the difference in the temperature of the room from month to month which would influence the rate of melting. Two mixes, one containing frozen condensed skimmilk and the other fresh condensed skimmilk, made the same day, frozen the same day, and the melt down tests conducted at the same time gave almost identical results, although significantly different in heat coagulation time and viscosity and similar in other properties studied. This would indicate that the measurements made have no relation to the melting characteristics or the variation in temperature of melting overshadowed differences due to condensed milk ingredients.

## DISCUSSION OF RESULTS

The chemical analysis of the milk supply used in the manufacture of the condensed skimmilk showed that the milk was normal in regard to per cent of solids, milk fat, lactose, total protein, and ash.

No definite relationship was found between the acidity of the milk products as indicated by the titratable acidity and pH and the protein stability as determined by the alcohol number and the phosphate coagulation time. Rice and Markley (9) found no relationship between natural acidity and coagulability with rennet or alcohol. They did find that these properties ran somewhat hand in hand and they concluded that both depended upon certain relationships between constituents of milk which are independent of acidity. Holm, Webb, and Deysher (2) did not find any correlation between stability of the protein of the fresh milk toward heat and that of the condensed milk manufactured from it. Our results indicated that the heating of whole milk such as that occurring in the process of pasteurization does lower the titratable acidity, due probably to the loss of $CO_2$. Whittier and Benton (12) observed a drop in the titratable acidity of milk upon heating and stated that the loss of $CO_2$ was the factor responsible. The reason for the increase in titratable acidity of the skimmilk upon condensing was no doubt due to the increased solids content.

In this study, no milk was found to produce a condensed skimmilk unstable to freezing and storing for a period of 4 weeks at $-17.8°$ C. (0° F.). Therefore, it was not possible to state the alcohol number or minimum coagulation time in the phosphate test which would differentiate between the condensed skimmilk suitable for freezing and storing and that skimmilk which would not be suitable because of the instability of the protein fraction.

The freezing and storing of the condensed skimmilk did not alter its physical appearance or body other than to make it slightly sandy. However, as shown in table 2, some changes did occur in the chemical properties. It may be seen that freezing and storing did cause an increase in titratable acidity. The fact that this increase was not consistent may have been due to the original whole milk as well as the condensed skimmilk. It is also rather difficult to explain the drop in pH which occurred in 8 of the 9 lots of condensed skimmilk. A lowered pH would indicate a reduced charge on the protein particle which would reduce the stability somewhat. However, with one exception, the protein stability of the condensed skimmilk as measured by the alcohol test of Dahle and Pyenson (1) was affected only slightly by storing at 0° F. for 4 weeks.

There was no significant trend found in the titratable acidity, pH or viscosity of the ice cream mix due to the changes produced by freezing and storing the condensed skimmilk at 0° F. Winn (13) found that the pH and titratable acidity of the ice cream mixes made from the stored frozen condensed skimmilk were not significantly different from those of ice cream mixes made with the same condensed skimmilk when fresh.

Fluctuations obtained in whipping ability as measured in terms of time to reach 100 per cent overrun do not permit the conclusion that the use of stored condensed skimmilk had any consistent effect on this property. The

score given the ice cream containing the fresh condensed skimmilk agreed very closely to that given the ice cream containing the same condensed skimmilk after it had been stored frozen for 4 weeks at 0° F. This indicated that from the standpoint of body and texture, as well as flavor, condensed skimmilk could be held frozen in storage for 4 weeks at 0° F. and still be used to produce a quality ice cream.

In the measurement of heat stability it was found that 6 of the 9 pairs of mix studied showed less stability when the stored product had been used. Information was not obtained which would explain why that did not hold true in the other 3 pairs of mixes studied. It is also difficult to explain the widely varying results obtained when the ice cream samples were melted down, unless attributed to the fact that the temperature at which the melt down tests were conducted usually varied for each mix of the pair being compared.

It must be recognized that the physical and chemical properties of the skimmilk and cream used in these ice cream mixes may have influenced the properties of the mixes studied.

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Burgwald, L. H.
Bushnell, L. D.

Cole, W. C.
Cone, J. F.
Corbett, W. J.
Coulter, S. T.

Doan, F. J.

Dorsey, L. M.
Downs, P. A.

Erb, J. H.
Espe, D. L.

Frazier, W. C.

Garrett, O. F.
Glick, D. P.
Goss, E. F.

Hansen, Arne
Huffman, C. F.

Irvine, O. R.

Josephson, D. V.

Knight, D.

Lucas, P. S.
Lush, J. L.

Mack, M. J.
Macy, H.
Marquardt, J. C.
Martin, W. H.
Mueller, W. S.

Parfitt, E. H.
Price, W. V.

Reece, Ralph P.
Riddell, W. H.
Ritter, W.

Stark, C. N.
Stebnitz, V. C.

Thomsen, L. C.
Trout, G. M.
Tuckey, S. L.

Webb, B. H.
Weckel, K. G.
White, G. C.

Yale, M. W.

## JOURNALS

American Butter Review
American Milk Review
American Journal of Diseases of Children
American Journal of Physiology
American Journal of Public Health
Archives of Pediatrics

Biochemical Journal
Biochemische Zeitschrift

Canadian Dairy and Ice Cream Journal
Canadian Public Health Journal
Certified Milk
Cornell Veterinarian

Dairy Industries
Dairy World
Deutsche Molkerei Zeitung

Endocrinology

Food Industries
Food Manufacture
Food Research

Ice and Refrigeration
Ice Cream Field
Ice Cream Industry
Ice Cream Review
Ice Cream Trade Journal
Industrial and Engineering Chemistry

Journal of Agricultural Research
Journal of Agricultural Science
Journal of American Veterinary Medicine Association
Journal of Bacteriology
Journal of Biological Chemistry
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Journal of Dairy Science
Journal of Endocrinology
Journal of Experimental Medicine
Journal of General Physiology
Journal of Heredity
Journal of Genetics
Journal of Infectious Diseases
Journal of Milk Technology
Journal of Nutrition
Journal of Pathology and Bacteriology
Journal of Physical Chemistry
Journal of Physiology

Kaeseindustrie
Kolloid-Zeitschrift

Lancet
Le Lait

Milchwirtschaftliche Forschungen
Milchwirtschaftliche Zeitung
Milk Dealer
Milk Industry
Milk Plant Monthly
Molkerei Zeitung
National Butter and Cheese Journal

Oil and Soap

Pacific Dairy Review
Proceedings of Society of Animal Production
Proceedings of Society of Experimental Biology and Medicine

Refrigerating Engineering

Scientific Agriculture

Tierernährung
Tierzüchter

Zeitschrift für Infektionskrankheiten Parasitäre Krankheiten und Hygiene der Haustiere
Zeitschrift für Physikalische Chemie, Abt. A and B
Zeitschrift für Untersuchtung der Lebensmittel
Zeitschrift für Züchtung. Reihe B. Tierzuchtung und Zuchtungbiologie
Zentralblatt für Bacteriologie
Züchtungskunde

## SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland

International Association of Ice Cream Manufacturers
International Association of Milk Dealers

National Institute for Research in Dairying, Reading, England
New York Association of Dairy and Milk Inspectors

Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copehagen, Denmark

United States Department of Agriculture

# ABSTRACTS OF LITERATURE

ADVANCE ABSTRACTS OF REPORTS TO APPEAR IN THE JOURNAL OF DAIRY SCIENCE

**383. Effect of Salt on the Keeping Quality of Cream.** W. J. CAULFIELD, F. E. NELSON AND W. H. MARTIN, Kansas Agricultural Experiment Station.

Four lots of 30 per cent cream to each of which salt in quantities equal to 0, 7, 10, 13 and 16 per cent of the weight of the fat-free serum was added immediately after separation, were held at 60, 70, 82 and 90° F. for 10-day periods. Changes in acidity, formol titration and grade were followed. Changes in the bacterial flora of two of the four lots were followed by direct microscopic observations.

Deterioration of the creams was definitely retarded by the addition of salt. The amount of salt necessary to effectively prevent appreciable deterioration was found to be dependent upon time and temperature of storage. The results indicate that not less than 10 per cent salt (serum basis) is required to prevent cream from becoming second grade when held for 10 days at 60 or 70° F. and for 5 days at 82 and 90° F.

The addition of 13 per cent salt (serum basis) to cream held at 70° F. for 3 or more days before salt was added did not prevent further deterioration of the cream. Thus the method is largely limited to farm use.

Butter churned from cream to which 13 per cent salt (serum basis) was added at the beginning of a 10-day storage period at 70° F. scored two to five points higher than did butter produced from control lots of the same cream held under similar conditions without salt.

When a modified Babcock test procedure was used, results which compared favorably with the calculated butterfat percentages were obtained.

The data indicate that the improvement of the keeping quality of cream by the addition of salt has definite merit.

**384. The Effect of Alfalfa Lipids upon the Progress of Sweet Clover Poisoning in Cattle.** W. A. KING, H. A. CAMPBELL, I. W. RUPEL, P. H. PHILLIPS AND G. BOHSTEDT, Departments of Biochemistry and Dairy Husbandry, University of Wisconsin, Madison.

A study of the effect of alfalfa lipids upon the progress of sweet clover poisoning in cattle has been made and the following results obtained.

Ten per cent of the ration of growing cattle was made up of toxic sweet clover and fed without harm for a period of $3\frac{1}{2}$ months.

Animals with a prolonged clotting time developed an increased number of blood platelets. There was no change in the fibrin, hemoglobin, or serum calcium in these cases.

Crude petroleum ether extracts of alfalfa hay fed at a level equivalent to 60 per cent of the toxic sweet clover in the ration brought about a favorable remedial response in sweet clover poisoned young cattle. Evidence adduced from the separate effects upon whole blood clotting time and prothrombin clotting time, the administration of bile salts alone and with alfalfa lipids, and the difference in rate of return to normal between the prothrombin and blood clotting times when the toxic hay was withdrawn from the ration indicates that one or more factors other than prothrombin were involved in the restoration of the normal blood clotting mechanism of the sweet clover poisoned bovine.

**385. The Effect of Added Egg Phospholipids on the Nutritive Value of Certain Vegetable Oils.** E. J. SCHANTZ, R. K. BOUTWELL, C. A. ELVEHJEM AND E. B. HART, Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

It was recently reported from this laboratory[1] that butter fat has a higher nutritive value for growth in weanling rats than certain vegetable oils when homogenized into mineralized skimmed milk and supplemented with all the known essential fat soluble vitamins. The difference in the nutritive value was not found to be due to factors contained in the non-saponifiable fraction of butter fat.[1] However, since the phospholipids are decomposed upon saponification it appeared possible that the difference in the nutritive value of butter fat and the vegetable oils fed might be due to some particular phospholipid contained in the butter fat which was not contained in the vegetable oils.

Addition of 0.25 per cent and 0.5 per cent of egg lecithin to corn oil or coconut oil improved the nutritive value of these oils slightly but not enough to make them equal to butter fat when they were homogenized into mineralized skimmed milk at a level of 4 per cent and fed to weanling rats.

Sphingomyelin sphingosine sulfate, and ethanolamine had no effect on the nutritive value of corn oil, but choline, in the case of the females, seemed to improve it slightly.

**386. The Nutritive Value of the Fatty Acid Fractions of Butter Fat.** E. J. SCHANTZ, R. K. BOUTWELL, C. A. ELVEHJEM AND E. B. HART, Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

In a previous paper[1] it was reported that butter fat homogenized into mineralized skim milk gave better growth of weanling rats than certain

[1] Schantz, E. J., Elvehjem, C. A., and Hart, E. B. The comparative nutritive value of butter fat and certain vegetable oils. J. DAIRY SCIENCE **23**, 181, 1940.

vegetable oils homogenized into skim milk and fed under the same conditions with ample carotene, irradiation, $\alpha$-tocopherol and minerals added in all cases. The superiority of butter fat for growth was not found to be due to factors contained in the non-saponifiable fraction of butter or to be due to compounds such as lecithin, choline, sphingomyelin, or sphingosine.

The fatty acids responsible for the superior growth of young rats obtained on butter fat as compared with certain vegetable oils homogenized into skim milk with all of the known essential fat soluble vitamins added, apparently lie in the saturated fraction of butter fat.

When the fatty acids of butter fat were separated into the volatile acids by steam distillation and into the unsaturated and saturated acids as lead soaps and the triglycerides of these fractions fed in corn oil in approximately the composition found in butter the *saturated* fraction with corn oil was found to be a little superior to butter fat while the other two fractions compared favorably with corn oil.

**387. The Length of the Intestine of Calves and Its Bearing on the Absorption of the Nutrients from the Chyme.** DWIGHT ESPE AND C. Y. CANNON, Iowa State College, Ames, Iowa.

Data are presented indicating that the small intestine of the living calf is about seven times the body length, or about one-third the post-mortem length. The large intestine does not show as great a difference in length between the living and post-mortem stages as does the small intestine.

Variations in the ratio between body length and length of intestine depend more on individuality than upon the age of the calf.

**388. The Specificity of the Lactogenic Hormone in the Initiation of Lactation.** A. J. BERGMAN AND C. W. TURNER, Dept. Dairy Husbandry, Univ. of Missouri, Columbia, Missouri.

A study is reported with the "lactogenic hormone" and the "thyreotropic and other hormone" fractions of the anterior pituitary on lactogenesis in the pseudo-pregnant rabbit. The injection of small amounts of the "lactogenic hormone" fraction was necessary to produce abundant lactation, (+++) to (++++) glands. Extracts rich in the "thyreotropic and other hormones," but containing only traces of the lactogenic hormone did not possess the ability to initiate lactation in doses as high as could be tolerated. These results are taken to indicate that the primary function of the lactogenic hormone, which also possesses the ability to proliferate the pigeon crop gland, is to initiate and maintain established lactation, while the "thyreotropic and other hormones" fraction has only a supplementary effect on established lactation.

**389. Distribution of *Pseudomonas fragi*.** H. B. MORRISON AND B. W. HAMMER, Iowa State College, Ames, Iowa.

*Pseudomonas fragi* was found in 16.5 per cent of 176 lots of milk delivered by 14 producers to an Iowa milk plant. It was not detected in 17 lots delivered in June to a Kentucky milk plant but was found in 40.0 per cent of 40 deliveries in December to the same plant. Samples of defective dairy products, especially those criticized as rancid or as having a May apple odor, commonly yielded the organism.

In general, dairy plant equipment was relatively free of the organism. An appreciable percentage (9.7 per cent) of the Iowa dairy plant water supplies yielded the organism. In Iowa a large proportion (51.8 per cent) of samples of dirt and other materials or equipment likely to come in contact with or be contaminated by dirt was found to harbor *Ps. fragi*. In Kentucky relatively few (4.1 per cent) similar samples obtained in summer yielded it, but it was found in a considerable proportion (37.4 per cent) of the samples obtained in December.

*Ps. fragi* was found in 25 (71.4 per cent) of 35 samples of barnyard soil obtained from various states. It was present in a larger percentage of samples from the eastern states (90.9 per cent) than from the western states (38.5 per cent).

The wide distribution of *Ps. fragi* on farms emphasizes the importance of farms as a source of the organism.

**390. A Study of Fresh and Frozen Plain, Superheated and Sweetened Condensed Skimmilk for Ice Cream.** L. K. CROWE AND HARRY H. WINN, Dairy Husbandry Department, University of Nebraska, Lincoln, Nebr.

Plain, superheated and sweetened condensed skimmilk made from the same lot of skimmilk was used fresh and after one, two, and three months storage at 0° F. as the source of added serum solids in ice cream. The three types of condensed skimmilk were satisfactory sources of serum solids when fresh and after storage. No benefits were derived from superheating condensed skimmilk that was stored frozen. The protein in superheated condensed showed precipitation after one month at 0° F. Storage of any of the three types of condensed skimmilk had no consistent appreciable effect upon the protein stability of the ice cream mix in which they were used.

Viscosity of the ice cream mixes was not consistently affected by the freezing and storing of the condensed skimmilk. The viscosity of the ice cream mixes made with superheated condensed skimmilk was but slightly higher than that of ice cream mixes made with the other two types of condensed skimmilk.

Average whipping curves indicate that ice cream mixes made with fresh plain condensed skimmilk whipped to 100 per cent overrun faster than ice

cream mixes made with fresh condensed skimmilk of the other two types. The time required to reach 100 per cent overrun for ice cream mixes made with the three types of condensed skimmilk stored frozen two and three months was less than the time required for mixes made with fresh condensed skimmilk.

There was no appreciable difference in the flavor, body or texture scores which could be attributed to the type of condensed skimmilk used or whether it was the fresh or frozen product.

Ice cream made with fresh superheated condensed skimmilk was slower in melting than ice cream made with either of the other two types of condensed. Ice cream made with superheated condensed skimmilk gave the least foam on melting. Freezing and storing plain and superheated condensed skimmilk increased the amount of foam in the melted ice cream whereas the opposite was true for sweetened condensed skimmilk. Ice cream made with stored frozen superheated condensed skimmilk was frequently criticized for a slight curdy appearance on melting.

**391. A Semimicro-Kjeldahl Method for the Determination of Total Nitrogen in Milk.** S. G. MENEFEE AND O. R. OVERMAN, Univ. of Illinois, Urbana, Ill.

Recent research work in this laboratory pertaining to the nitrogen distribution in dairy products made it desirable to develop a practical method for determining small amounts of nitrogen.

The purpose of this experiment was: (1) to design a semimicro-Kjeldahl apparatus, (2) to compare the efficiency of several digestion catalysts and (3) to compare both 0.02 N sulphuric acid and boric acid solutions as the ammonia receiving agents.

Copper sulphate and selenium oxychloride, mercuric oxide and selenium oxychloride, and mercuric oxide alone were used as the digestion catalysts. These three catalysts gave comparable results when used to analyze milk for total nitrogen. Mercuric oxide is preferred as a catalyst because the literature for the most part, indicates that selenium and its combinations cause low results for nitrogen.

A 2 per cent boric acid solution is preferred as the ammonia receiving agent with a methyl red-methylene blue combination as the indicator. The boric acid solution eliminates the use of 0.02 N sodium hydroxide solution (for back titrating the standard acid) which is very sensitive to carbon dioxide and requires frequent restandardization.

The semimicro-Kjeldahl method is well adapted to routine analysis, it saves time, and reduces the cost of reagents per determination.

The results obtained with the semimicro-Kjeldahl check very closely with those obtained by the Official Method.

## BOOK REVIEWS

**392. Food Industries Manual; 10th Edition.** Published by Leonard Hill Limited, London. Distributed in U. S. by Chemical Publishing Co., Inc., New York. 400 pages, price $4.00.

This is a technical and commercial compendium of the manufacture, preserving, packing and storage of various food products. The volume aims at collecting information which has become established and standardized in the various food industries. It succeeds best in doing this in the case of milling, baking, sugar confectionery, chocolate, and canning. The section devoted to the dairy industry is limited and chiefly contains formulae for various common dairy calculations, some test procedures and a few tables on the composition of dairy products. The volume contains an extensive list of books likely to interest those engaged in the food process industries, but it has not been brought up to date. The subject matter is so broad that it has been necessary to condense greatly most of the subjects dealt with.

J.H.E.

**393. Dairy profit.** WILBUR J. FRASER, Interstate Printers and Publishers, Danville, Ill. 270 pages, Illustrated. Price $1.80. 1940.

"Dairy Profit" is a valuable contribution to dairy literature.

Although much of the material has appeared previously in periodicals, it is here grouped and classified and Professor Fraser has added much of his own philosophy about agriculture and especially dairying and dairy people.

The author emphasizes the need for planning farm and dairy operations on a long time basis.

The subject matter of the book deals with the problems of feeding and management and crop production necessary to successful dairy farm operation.

Professor Fraser discusses the principles of feeding and management in simple straight forward terms and in most instances illustrates the effect of good management by a story of some good farmer. Throughout the book he stresses the need of proportionality and shows how disastrous it is to do part of the job well in all particulars save one and then miss the goal of success.

The word "profit" in the title is used in its broadest sense for the author in the last chapters of the book shows the need of planned recreation. He discourages the unnecessary drudgery which may be needed to merely accumulate large holdings of land and money. This idea is indicated by the title of one of the chapters, "What Shall it Profit a Man."

This book should be of special interest to farmers and teachers; to farmers, it gives suggestions and encouragement; to the teachers it will give a wealth of illustrations to vitalize subject matter. C.L.Blackman

# BACTERIOLOGY

**394. The Action of Chemical Disinfectants on Bacteriophages for the Lactic Streptococci.** G. J. E. HUNTER AND H. R. WHITEHEAD. The Dairy Research Institute, Palmerston North, New Zealand. J. Dairy Res., *11:* 62–66. 1940.

The times needed for the complete inactivation of bacteriophages for the lactic streptococci by hypochlorite, potassium permanganate, hydrogen peroxide, formaldehyde, mercuric chloride, alcohol, and phenol in various strengths determined. The phage preparations consisted essentially of whey. Inactivation was determined by failure of the phage chemical mixture to prevent the coagulation of milk inoculated with the susceptible organism.

When all the phage preparations were adjusted to a common protein percentage of 0.49 per cent, it was found that all of the eight phage races studied were destroyed by exposure to 0.05 per cent active chlorine within 1 minute. Active chlorine and permanganate were by far the most effective of the disinfectants.

Hydrogen and hydroxyl ions were found to inactivate phage when they were present in sufficient concentrations, but their effects between pH values of 4 and 7 were negligible. S.T.C.

**395. The Influence of Various Factors on the Fermentation End-Products of the Heterofermentative Streptococci.** C. C. THIEL, The Nat'l Inst. for Res. in Dairying, Univ. of Reading, Reading, England. J. Dairy Res., *11:* 51–61. 1940.

The influence of temperature, pH, oxygen tension and yeast autolysate on the production of by-products and the ratios of the by-products formed to the sugar utilized and lactic acid produced in milk by the heterofermentative lactic acid streptococci were investigated.

The total production of lactic acid was increased by anaerobic conditions and low temperature. The ratio of lactic acid formed to sugar utilized was increased by anaerobic conditions and the presence of chalk.

The total amount of acetic acid was higher in the presence of chalk at the lower temperatures and in yeast milk, but was decreased by anaerobic conditions. The ratio of acetic acid both to sugar utilized and to lactic acid formed was smaller at lower temperatures, in the presence of "growth factors" and chalk and under anaerobic conditions.

The total alcohol production was high when yeast or chalk was added to milk, under anaerobic conditions and at lower temperatures, and similarly the ratio of alcohol formed to sugar utilized and lactic acid produced was increased.

Hydrolysis of the residual lactose occurred in all cultures of *Str. citrovorus* and with some of the other streptococci in cultures to which chalk had been added. S.T.C.

**396. Studies on the Methylene-Blue Reduction Test. II. Comparison between the Old and the Modified Methods.** T. MATUSZEWSKI AND J. SUPINSKA, Institute of Fermentative Industry and Agricultural Bacteriology, Warsaw, Poland. J. Dairy Res., *11:* 43–50. 1940.

The old and modified (inversion of tubes at 30 minute intervals) methods of the methylene blue reduction test were compared using 185 samples of raw milk. The deviations in the reduction time, corresponding to a given initial number of cells, are smaller by the modified than by the old method, and the average reduction time is shorter. It is suggested that this is due to a more uniform distribution of the bacteria in the milk following the inversion of the tube.

The average coefficient of multiplication in the old method was 0.660 and in the modified method 0.885, showing that the shortening of the reduction time in the modified method is due to stimulation of bacterial growth. S.T.C.

**397. Haemolytic Organism Isolated from Pasteurized Cream.** L. O'DROMA, Dept. of Dairy Bacteriology, University College, Cork, Ireland. J. Dairy Res., *11:* 37–42. 1940.

A description is given of a weakly haemolytic organism, which first appeared as a contaminant, in the form of pin point colonies, in agar plates inoculated with dilutions of a cream which had been subjected to partial processing in a butter churn. The majority of its characteristics showed it to be a resistant strain of *Str. thermophilis,* although it resembled in many respects certain strains of *Str. Bovis.* S.T.C.

## BREEDING

**398. Rindviehzucht in Schweden.** IVAR JOHANSSON. Züchtungskunde *15* (4) : 97–104. 6 figs. 1940.

Three breeds of cattle are bred pure in Sweden. The Black and White which is similar to the Holstein-Friesian is most abundant in southern Sweden. The Red and White which is somewhat like the Shorthorn and Ayrshire is the most popular in middle Sweden. A smaller hornless native race is more abundant in northern Sweden. Pictures of the Red and White and of white hornless animals are included. Cow testing associations began in 1898. About 18 per cent (350,000 head) of the milking cows are now on test in these associations. About 50,000 other cows are on test in creamery

associations where the owner weighs the milk and takes the samples himself but the testing is done at the nearest creamery. These creamery tests are not recognized in the conduct of the herdbooks or when awarding prizes at the fairs but are thought worth making for the owner to use in controlling his feeding operations and in culling. Progeny tests of bulls generally require at least ten comparisons between daughters and dams but in certain circumstances six such comparisons justify evaluating a bull. Some government aid is given to the cow testing associations and also to the 2,400 bull associations. Members of the latter own 286,000 cows. There are no specialized beef breeds in Sweden and no cattle are now used for draft purposes. Breeding is aimed at a milk-flesh ideal with the milk being given more emphasis, especially in the northern districts. The total number of cows has not changed much in recent years but production has increased. Improved breeding and better management have both been responsible for this. About eight per cent of the feed for dairy cows is imported. Various governmental subsidies or control measures have kept the domestic price for butter higher than the price for butter exported. Some of these last circumstances may have changed since the outbreak of the present war.

J.L.L.

**399. Die Auswertung der Herdbücher in den Fleckviehzuchtgebieten unter besonderer Berücksichtigung der Leistungsvererbung durch den Bullen.** HANS BIEGERT. Züchtungskunde *15* (4) : 105–119. 1940.

The author gives first a general discussion about the purposes and uses of herdbooks, the nature of the data they do or should include, methods of evaluating inheritance for milk production, etc. The data studied are from the herdbooks of the Ried region in central Baden and involve evaluating the production or inheritance of about 140 bulls and 4,000 cows. All of the cattle belong to the Fleckvieh race (similar to the Simmenthaler). The agriculture of this region is characterized by small holdings with usually not more than three or four cows per farm and consequently the extensive use of community bulls and such cooperative measures. Under such conditions the author believes it unwise to refer each record to the herd average, as has been recommended for regions where the herds are very large and the data extend over many years. Also he concludes that the year-to-year variation need not be considered in types of agriculture in which little use is made of pasture. Daughter and dam were tested in the same herd in about 95 per cent of the cases. Age corrections are avoided by not including the first two lactations in the daughter-dam comparisons. He uses averages of the third and fourth lactations and later ones, if any. Each sire is evaluated according to the customary "heredity grid" diagram which is like an intrasire daughter-dam correlation. The diagram also shows the numbers of the

daughters which exceed or fall below their dams and the number which exceed or fall below the average for the community in which that bull was kept. Daughters without tested dams or without third and fourth lactations are indicated in the diagram but are not included in the proof. Ten daughter-dam comparisons are considered the minimum for proving a sire. Corrections for the amount of draft work performed by the dam or by the daughter are discussed but are considered unsatisfactory. An actual pedigree is given as a model of what would be expected in pedigrees giving only the most useful information. No new genetic theory is involved. This is a discussion with actual examples of what can be done toward the proving of dairy sires in communities where the herds are small and the cooperative use of dairy sires is extensive. It also includes a number of brief statements of opinion as to which external circumstances are practically worth correction and concludes that in this region age is almost the only one of these.

J.L.L.

## BUTTER

**400. Crumbly, Sticky Butter.** C. H. PARSONS, Swift & Co., Chicago. Nat'l Butter and Cheese J., *31:* 4, 18. 1940.

Crumbly butter is difficult to cut into patties and breaks easily when cold. Composition of butter fat and manufacturing procedures influence the extent of the defect. Butter manufacturers cannot control feeds but may relieve the condition by careful manufacturing methods. Control of temperature of churning and temperature of wash water as suggested by Coulter and Combs were tried on the commercial scale in 9 states. Butter was tested by penetrometer, and by cutting and spreading tests. The treatments improved but did not wholly overcome severe crumbly conditions. The auger type printing machine improved body. There is a possibility of further improvement by proper adjustment of time and temperature of holding butter before printing.

W.V.P.

**401. Weedy Flavored Cream—Its Relation to the Butter Industry.** P. A. DOWNS, Univ. of Nebraska, Lincoln, Nebr. Nat'l Butter and Cheese J., *31:* 5, 12. May 1940.

Flavors of milk and cream may come from feeds and weeds eaten by cows or from odors breathed by the cow and absorbed by the blood stream. *Thalaspi arvense,* called Pennycress, Frenchweed or stinkweed, belongs to the mustard family and now is found from Canada south to central California and east to eastern Minnesota. As little as 90 to 150 gms. of seed or 500 gms. of green forage eaten by the cow will taint the milk. Wild onion or garlic plants are common causes of tainted milk. "Pepper grass" is believed to cause a characteristic flavor but more definite information is

needed to be sure of its effect. This information is being obtained by the combined efforts of the Research Committee of the American Butter Institute of Chicago, and a Sub-committee of the Quality Committee of the American Dairy Science Association. W.V.P.

## CHEESE

**402. The Measurement and Significance of pH Values in Cheesemaking.** J. G. DAVIS AND C. C. THIEL, The Nat'l Inst. for Res. in Dairying, Univ. of Reading, Reading, England. J. Dairy Res., *11:* 71–78. 1940.

A colorimetric dilution method for the determination of pH values in cheesemaking is recommended, using bromthymol blue as the indicator for milk and whey of pH greater than 5.8 and B. D. H. 5560 indicator for whey of pH below 5.8. The authors consider that titratable acidities and pH values each afford an incomplete picture of the working of a curd. Together they afford a valuable indication of the normality or otherwise of the making process. S.T.C.

**403. Cream Cheese as a Base for Spreads.** C. R. BAKER. Nat'l Butter and Cheese J., *31:* 6, 26. June 1940.

A method of making "Cream cheese" with six per cent fat in the mix is outlined. The curd may be mixed with ripened cheese, pickles, olives or pimentos. Emulsifying salts and a stablizing agent are added to the cheese mixture which is cooked to about 170° F. before placing in glass jars. W.V.P.

## CHEMISTRY

**404. Biennial Reviews of the Progress of Dairy Science.** Section C. Dairy Chemistry. J. Dairy Res., *11:* 84–111. 1940.

An excellent review of the progress of dairy chemistry from the middle of 1937 to the middle of 1939. S.T.C.

**405. The Determination of Vitamin D in Food Substances Containing Phosphorus.** KATHARINE COWARD AND ELSIE KASSNER, College of Pharmaceutical Society, London. Biochem. J., *34:* 538. 1940.

In this paper a reinvestigation of the effect of giving vitamin D plus phosphate to rats fed on a rachitogenic diet of high-Ca, low-P content was reported. In six separate experiments the average healings produced by (a) 10 units of vitamin D, (b) a dose of potassium phosphate, and (c) 10 units of vitamin D plus a dose of phosphate were compared. The phosphate dose of groups (b) and (c) ranged from 60 to 1380 mg. per rat. In another series

similarly arranged the vitamin D dose was 5 units, and the phosphate dose ranged 460, 920 and 1380 mg. per rat. The lower doses of phosphate alone were practically without effect on calcification but all doses affected the calcification brought about by either 5 or 10 units of vitamin D. The curves of response to (a) 10 units of vitamin D plus graded doses of phosphate, (b) 5 units of vitamin D plus graded doses of phosphate, and (c) doses of phosphate alone were all logarithmic and of similar slope, (a) being highest, and (c) lowest of the various heighths. Giving both vitamin D and phosphate to rats receiving a rachitogenic diet of high-Ca, low-P content has a multiplicative effect on healing, not an additive one. It is suggested the only practical method of testing a food product containing sufficient phosphorous in the dose to alter the Ca-P ratio of the diet is to extract the ether soluble portion after saponification and determine the vitamin D in the extract.

K.G.W.

**406. Studies on the Chemistry of the Fatty Acids. VI. The Application of Crystallization Methods to the Isolation of Arachidonic Acid, with a Comparison of the Properties of this Acid Prepared by Crystallization and by Debromination. Observations on the Structure of Arachidonic Acid.** G. Y. Shenowara and J. B. Brown, Lab. of Physiological Chem., Ohio State Univ., Columbus, O. J. Biol. Chem., *134:* 331. 1940.

Crystallization procedures at low temperatures were applied to the methyl esters from the fatty acids of suprarenal phosphatides. The methyl arachidonate obtained by this method was compared, for the constants, with the ester prepared by the debromination procedure. The differences in constants observed are believed due to the presence of isomers in the product obtained by the chemical means. A tentative formula for the arachidonic acid is suggested, based on physico-chemico measurements. The arachidonic acid occurring in adrenal phosphatides is suggested as $\triangle$-6-10-14-18 $\alpha$ icosatetrenoic acid.

$CH_3 - CH = CH - (CH_2)_2 - CH = CH - (CH_2)_2 - CH = CH - (CH_2)_4 - COOH$. This information is of interest because of increasing attention to the value of fatty acids in nutrition. K.G.W.

**407. The Estimation of Riboflavin. 1. A new biological method. 2. The estimation of riboflavin in milk; comparison of fluorimetric and biological tests. 3. Statistical analysis of the data.** M. M. El Sadr, T. F. Macrae and C. Elizabeth Work, Div. of Nutrition, Lester Institute, London; K. M. Henry, J. Houston and S. K. Kon, Nat'l. Institute for Research in Dairying, Univ. of Reading; and J. O. Irwin, Medical Research Council's Statistical Staff. Biochem. J., *34:* 601. 1940.

The first phase of the paper is a concise review of the techniques available for measuring the riboflavin content of foods, and the results of these methods. The second phase is a report of a study on the biological assay method wherein a basal ration plus various supplements were fed the animals. A method was suggested for use whereby young rats are fed a diet complete in other respects plus a liver charcoal filtrate as a source of the $B_2$ vitamins. Inasmuch as the rats showed growth responses when fed graded doses of riboflavin, it is believed an excellent method for assay purposes.

In the third phase a comparison was made between the fluorimetric method of measurement of riboflavin and the bioassay method. When the milk is fed at lower levels yielding animal-weight gains of 10–15 grams a week, biological findings are in good agreement with fluorimetric tests. In the fourth phase, the bioassay results of two laboratories were put to statistical review. The results of the data show that the slope of the standard curve of data by the one laboratory is steeper than the slope for standard of the second laboratory. Because different slopes for the standard curve occur in different laboratories, it is suggested that at least two doses of standard and two doses of unknown preparation be employed in routine testing. K.G.W.

**408. The Composition of Dolphin Milk.** LILLIAN EICHELBERGER, E. S. FETCHER, JR., E. M. K. GEILING AND B. J. VOS, JR., Lasker Foundation for Medical Research and the Depts. of Medicine, Pharmacology, and Physiology, Univ. of Chicago. J. Biol. Chem., *134:* 171. 1940.

The data presented in this study were obtained in a manner unlike that employed for other studies; the samples were obtained from *live* animals, three bottle-nose dolphins and in addition, one spotted dolphin 1.5 hours after it had been harpooned. Dolphin milk was found to be high in fat (108–180 gm. per liter) and protein (94.2–111 gm. per liter) and low in lactose (3.9–7.7 gm. per liter). K.G.W.

**409. The Determination of pH in Milk and Whey by Means of Colour Indicator Paper.** L. SEEKLES, The Laboratory for Veterinary Chemistry, State University, Utrecht, Holland. J. Dairy Res., *11:* 79–83. 1940.

The author suggests the use of lypan paper M25 for pH measurements in milk and whey in the pH range 5.4–6.6. As a rule the error was found not to exceed 0.1 pH. In very acid milk and whey ($pH < 5.4$) lypan paper of the L. series is recommended. Generally the deviation was found not to exceed 0.2 pH. Lypan paper can not be used in alkaline milk and whey. S.T.C.

**410. A Rapid Method for the Separation of Serum Albumin and Globulin.** GEORGE B. KINGSLEY, Div. of Biochem., Labs., Phila. Genrl. Hospital, Phila., Pa. J. Biol. Chem., *133:* 731. 1940.

A rapid, accurate method for the separation of serum albumin and globulin is based on the use of ether to decrease the density of globulin precipitated by sodium sulphate. After ether extraction and brief centrifugation, globulin separates in a compact layer at the bottom of the ether phase above the sodium sulfate solution. No preliminary period of standing is required. K.G.W.

**411. Composition of Lactic Acid and the Production of a Highly Concentrated Acid.** PAUL D. WATSON, Res. Labs., Bur. Dairy Ind., U. S. Dept. of Agr. Ind. Eng. Chem., *32:* 399. 1940.

Lactic acid with a total acidity of about 100 per cent consists of varying proportions of lactic acid (free), lactyllactic acid (anhydride), lactide and water.

The composition of lactic acid of different strengths varies widely, and the composition of a single acid changes slowly until equilibrium is reached. Lactic acid (U.S.P. 85 per cent) contains only a trace of lactide; the 100 per cent acid contains about one per cent lactide. The amount of lactyllactic acid present may vary from about zero to about 90 per cent, dilute lactic acid containing little or none. Smooth curves are obtained when the analytical results obtained on stabilized acids are plotted.

At present concentrated lactic acid is not available in concentrations above 86 per cent. This study has resulted in a process for the production of concentrated lactic acid with a total acidity of about 105 per cent expressed as lactic acid. This concentrated acid is an anhydrous, viscous, water white liquid, and it is thought that it may have considerable industrial value. Author's Abstract

## CONCENTRATED AND DRY MILK; BY-PRODUCTS

**412. Density of Dry Milk Solids (Skimmilk).** O. E. STAMBERG AND C. H. BAILEY, Univ. of Minnesota, St. Paul, Minn. Food Research, *5:* 3, p. 275. May–June 1940.

The authors define a term "density index" as ten times the amount of sedimentation obtained, by shaking 7 grams of dry skimmilk with a naphtha and carbon tetrachloride mixture (density 1.250 at 25° C. or 77° F.) in a graduated 50 cc. conical centrifuge tube, after 45 minutes settling. Their observations indicate that there is a great variation in the density indices of spray dried skimmilk due to the variation in amount of occluded air. This in turn was found to vary with the process of manufacture and type of equipment. No air was found in roller dried skimmilk and less air was

found in spray dried skimmilk when a higher preheating treatment was used. The authors indicate that the density index might be used by manufacturers as a test for uniformity of product. While no mention is made directly concerning it, the index appears to also offer a means of distinguishing between spray dried and roller dried skimmilk. F.J.D.

## FEEDS AND FEEDING

**413. Milk and Butterfat Production by Dairy Cows on Four Different Planes of Feeding.** R. R. GRAVES, GEORGE BOTEMAN AND J. B. SHEPPARD, all of the Bureau of Dairy Industry, AND GEORGE B. CAINE, Utah State Agr. College. U.S.D.A. Tech. Bul. 724, 36 pages. April 1940.

Twelve Holstein cows were fed the following 4 different rations in 4 lactation periods and the production records calculated to maturity.

1. Full grain ration—consisting of alfalfa hay, corn silage, pasture in season and one pound of the grain mixture per pound of fat produced per week. Grain mixture was 2 parts barley, 1 part oats and 1 part bran.
2. Alfalfa hay alone or pasture alone in season.
3. Same as (2) except ground barley was fed at the rate of one pound to an average of 6.03 pounds of milk.
4. Alfalfa hay and pasture with the addition of corn silage.

Ration 1 was fed prior to the other rations which did not follow in order. On the basis of ration No. 1 being 100 per cent, ration No. 2 produced 69.75 and 65.77 per cent as much milk and butterfat respectively; ration No. 3 produced 86.03 and 80.24 per cent as much milk and butterfat respectively.

The average dry matter consumed daily per cow during the winter was 30.6 pounds on alfalfa alone, 32.85 pounds on alfalfa and restricted grain and 32.76 pounds on alfalfa and corn silage. W.E.P.

**414. Roughage Feeding of Dairy Cattle.** H. S. WILLARD, Univ. of Wyoming, Laramie, Wyoming. Bul. 237, May 1940.

Barley as the grain supplement to alfalfa hay and pasture was compared to alfalfa and pasture only for milking cows. The effect of several consecutive years of no grain feeding was also studied. From 2 to 12 pounds of barley per head per day was found to have very little effect upon lowering the hay consumption.

When Holstein cows had a daily capacity of 30 to 40 pounds of milk at the peak there was little increase in production when barley was fed with good hay and pasture. Cows with greater capacities at peak production benefit proportionally from grain feeding. When no grain is fed the greater the peak production capacity is, the more rapid the decline in daily milk production. Cows with 50 to 60 pounds peak production capacities produced

327 pounds fat in 305 days mature equivalent without grain and 410 pounds with barley supplement. Cows with 40 to 50 pounds daily peak capacities produced 317 and 320 pounds fat on the mature equivalent in 305 days for no grain and grain respectively. When the peak production capacity was 30 to 40 pounds the respective 305 day mature equivalent fat yield for no grain and grain was 240 and 214 pounds. W.E.P.

**415. Rate of Growth by Dairy Calves and Heifers on Different Rations.** R. R. GRAVES AND J. R. DAWSON, Bureau of Dairy Industry, Washington, D. C.; D. V. KOPLAND, Huntley, Mont.; A. G. VAN HORN, Lewisburg, Tenn.; and S. L. CATHCORT, Columbia, S. C. U.S.D.A. Cir. No. 560, 24 pages. 1940.

Holstein heifers were fed skim milk until 6, 12, 18 and 24 months of age. No difference was found in the rate of growth. The longer periods of skim-milk feeding resulted in a somewhat higher breeding efficiency. A slightly higher milk production was observed for the heifers fed skim milk for the longer periods which was not attributable to inheritance. At Woodward, Oklahoma, with 3 groups of 3 Holstein heifers each, it was found that satisfactory growth was secured on winter rations consisting of sumac sorgo silage and 1 pound cottonseed meal; sumac sorgo silage and 2 pounds cottonseed meal and 6 pounds alfalfa hay; sumac sorgo silage and 2 pounds grain mixture.

Jersey heifers were found to make satisfactory growth from 12 months of age when fed unlimited amounts of machine dried legume hay during the winter and good pasture during the summer. Thirteen Jersey heifers fed machine dried hay exclusively and without pasture from 12 months to 18 months of age made satisfactory gains. The daily hay consumption per head ranged from an average of 10.6 pounds at 13 months to 15 pounds at 18 months of age. W.E.P.

## FOOD VALUE OF DAIRY PRODUCTS

**416. A Physiological Explanation of the Therapeutic Value of Milk.** CHARLES F. NELSON, M.D., Nelson Clinic, Beverly Hills, Calif. The Assn. Bull. Intern. Assn. of Milk Dealers. 32nd year. No. 16: 393–398. May 1940.

The results of physio-chemical tests show that 68 per cent of all people are below 10 mgs. of blood calcium, whereas the author considers 10.5–12 mgs. per cent (equivalent to 12 mgs. per 100 ml. of blood) as the optimum. He further states that nearly all cows are deficient in calcium. The serum calcium and phosphorus determinations of 100 women on date of delivery showed 85 per cent were deficient in phosphorus. In order to maintain a normal calcium concentration in the blood, it is necessary to maintain a nor-

mal blood phosphorus concentration. Low blood calcium also results from too high phosphorus, in which event phosphorus intake must be reduced until the calcium-phosphorus serum concentration ratio is 3 to 1. If it is possible to increase the amount of calcium in cows' milk in addition to calcium containing foods, especially alfalfa, oats, etc., it is necessary to add some form of calcium medication to the food mixtures either in the form of decomposed limestone, calcium carbonate or calcium gluconate. A blood calcium content of 12 mgs. per cent is desired. E.F.G.

**417. The Vitamin A and Carotene Content of Shorthorn Colostrum.** K. M. HENRY, J. HOUSTON AND S. K. KON, Nat'l Inst. for Res. in Dairying, Univ. of Reading, Reading, England. J. Dairy Res., *11:* 1–8. 1940.

The concentration of carotene and vitamin A in colostrum and colostral fat and total yield of these substances in successive milkings was studied for four Shorthorn cows and nine heifers. The concentration of vitamin A in the first colostrum ranged from 8160 to 820 Moore blue units per 100 g. and that of carotene from 2026 to 411 Moore yellow units per 100 g. The highest and lowest concentrations and yields of vitamin A and carotene respectively in samples of colostrum and later milk were in the ratios: per g. of colostrum (milk) 35 : 1 and 65 : 1; per g. of fat 27 : 1 and 34 : 1; calculated on daily yield 31 : 1 and 65 : 1.

Access to pasture before calving appeared to have no effect on the secretion of vitamin A in colostrum but increased the output of carotene.

S.T.C.

**418. The Effect of Commercial Pasteurization and Sterilization on the Vitamin $B_1$ and Riboflavin Content of Milk as Measured by Chemical Methods.** J. HOUSTON, S. K. KON AND S. Y. THOMPSON, The Nat'l Inst. for Res. in Dairying, Univ. of Reading, Reading, England. J. Dairy Res., *11:* 67–70. 1940.

Fluorimetric tests applied to commercially pasteurized and commercially sterilized milk showed that in the former some 10 per cent and in the latter up to 50 per cent of vitamin $B_1$ was destroyed in the course of the heat treatment.

Riboflavin withstood both treatments without loss. S.T.C.

**419. The Problem of Variations in the Growth-Promoting Value of Milk for Rats.** S. BARTLETT, K. M. HENRY AND S. K. KON, The Nat'l Inst. for Res. in Dairying, Univ. of Reading, Reading, England. J. Dairy Res., *11:* 22–36. 1940.

The growth-promoting properties of milk from three Shorthorn cows grazed on the best available permanent pasture was compared to that pro-

duced by three other cows of similar lactational history which had been on winter rations for 6 months at the start of the experiment and continued to be stall-fed. By suitable blending of morning and evening milkings the fat content of the milk from both groups of cows was adjusted daily to a common level. A part of each milk was then holder-pasteurized.

Four groups of specially prepared litter-mate male rats were fed *ad lib.*, for a period of eight weeks, on raw and pasteurized milks, both being supplemented with minerals. There were no differences in appetite or gain in weight.

In similar experiments sugar was added to the milks to double their caloric value. Again the growth and appetite of the animals were the same on the raw summer and "winter" milks. Pasteurization did not alter significantly the value of the milks, but the summer milk, whether raw or pasteurized, was superior to the pasteurized "winter" milk.

Guinea pigs receiving the raw mineralized milks alone or with sugar died within a comparatively short time. There was no difference in growth performance or time of survival between summer and "winter" milk groups.

The authors state that these experiments give no indication of the presence of a specific new appetite or growth factor in pasture milk.

S.T.C.

**420. Influence of Age of Cow on Ascorbic Acid Content of Certified Milk.** A. D. HOLMES AND FRANCIS TRIPP, Research Labs., The E. I. Patch Co., Boston; E. A. WOELFFER, H. P. Hood and Sons, Boston; AND G. H. SATTERFIELD, Univ. of North Carolina, Raleigh, N. C. Food Research, *5:* 3, p. 263. May–June 1940.

As a result of the analysis of 659 samples of Guernsey and Holstein milk from animals stall fed on a variety of hays and concentrates, where the ages of the cows ranged from four to eleven years, no consistent relationship was noted between the age of a cow and the amount of ascorbic acid in the milk.

F.J.D.

## ICE CREAM

**421. Sugar in Ice Cream.** P. H. TRACY, Univ. of Illinois. Ice Cream Field, *35:* 5, 68. May 1940.

It is stated that approximately 200,000,000 pounds of sugar are used in the ice cream industry annually. The necessity of using sugar to insure palatability is emphasized and the influence of sugar upon freezing point lowering of ice cream mixes is indicated.

Mention is made of certain products, such as corn syrup or dried corn syrup which have recently been used in the ice cream industry. It is reported that experiments conducted by the author show that high conversion

corn syrup can be successfully used to replace from 50–75 per cent of sucrose in the manufacture of sweetened condensed milk. It is also stated that one-third to one-half the sucrose in ices or sherbets can be replaced by "Sweetose," this new corn syrup which was used in the experiments conducted by the author. It is claimed that this product improved the body and texture of ices and sherbets and enhanced certain fruit flavors, especially pineapple.

In ice cream when replacing 25 to 33⅓ per cent of sucrose with "Sweetose" on a sweetening value basis of two-thirds, no effect was noticed on mix color, pH or viscosity but the mixes whipped slightly slower. No deleterious effects were noted so far as flavor was concerned, but the body was improved according to the author. W.C.C.

**422. Vegetable Ice Cream.** VINCENT M. RABUFFO, Editor, Ice Cream Trade Journal. Ice Cream Trade J., *36:* 6, 12. June 1940.

Vegetable flavored ice cream has been developed and introduced by Philip Wenger, Tortoni Ice Cream Company, Newark, N. J. Tomato sherbet and spinach, carrot and fresh asparagus ice cream are among those sold. W.H.M.

**423. Soda Fountain Retail Ice Cream Stores.** W. L. MOLLOY, Sales Mgr., Grand Rapids Cabinet Co. Ice Cream Field, *35:* 5, 46. May 1940.

The wide range of conditions under which soda fountains are operated is pointed out. It is claimed that the deciding factor to consider when attempting to determine whether or not sandwiches, lunches, etc. should be served at the soda fountain is whether their introduction will increase the consumption of ice cream and other dairy products sold at the fountain. W.C.C.

**424. Texture in Ice Cream.** J. H. ERB, Ohio State Univ. Ice Cream Field, *35:* 6, 32. June 1940.

The author emphasizes the importance of low temperature drawing of ice cream from the freezer and rapid hardening as a means of improving ice cream texture. He claims that stiff ice cream from the freezer when hardened slowly at 0° F. was about equal in texture to soft ice cream from the freezer if the latter was hardened rapidly.

It is claimed that the ice cream from a continuous freezer compared to ice cream drawn at the same temperature from a batch freezer has a better texture because of the finer incorporation of air in the continuous freezer. W.C.C.

**425. Plant Maintenance.** F. C. VOGT, Pres., Vogt's Ice Cream Co., New York, N. Y. Ice Cream Field, *35:* 5, 50. May 1940.

According to the author each plant has its own specific maintenance

problems. This is especially true with refrigeration systems it is claimed; often lack of flexibility in such equipment makes the problem acute.

With other equipment, maintenance need not be difficult it is stated, since troubles can usually be forecast and proper repairs made before maintenance reaches the acute stage.

The necessity of properly grinding the valves in homogenizing equipment, of carefully handling sanitary piping, of maintaining proper strengths of cleaning solutions through regular and frequent checks, of operating the boiler and other pieces of equipment according to established practices are all mentioned as part of an efficient management program. W.C.C.

**426. Charting Better Retail Store Control.** ANONYMOUS. Ice Cream Field, *35:* 5, 29. May 1940.

It is claimed that analyses of many retail stores show lack of adequate and accurate control of merchandise and costs which is often a primary cause of failure. Two charts used by one successful ice cream company are reproduced to illustrate devices that can be helpful. These charts are entitled (1) "Efficiency Rating of Sales People," and (2) "These are Money Losses." They illustrate many points worthy of consideration. W.C.C.

**427. Retail Store Organization.** CHARLES PAINE, United Farmers Dairy Stores, Inc., Charlestown, Mass. Ice Cream Field, *35:* 5, 36. May 1940.

Proper organization according to the author divides the whole job into parts in such a way as to accomplish the desired results. Further, it defines each job as well as the inter-relationship between jobs thereby removing many causes of friction.

Merely assigning certain duties to each employee is not enough. It is claimed that organization necessitates that one (1) analyze or study the work to be done, (2) organize or group the various things to be done, (3) deputize or select proper personnel, (4) train each employee for his job, and (5) supervise or see that the program outlined is accomplished. W.C.C.

**428. Mechanical Refrigeration in Ice Cream Truck Bodies.** P. FORTNEY, Warnsman-Fortney Body Co., Cleveland, Ohio. Ice Cream Field, *35:* 5, 44. May 1940.

It is claimed that the most widely used type of mechanical refrigeration in the ice cream industry is the hold over system. This system consists of an evaporator coil which is immersed in or adjacent to a eutetic solution of silica jell. The compressor may be on the body or a remote installation. It is claimed that most buyers prefer the compressor on the truck body unless an ammonia compressor is used as the source of refrigeration.

This system according to the author maintains more uniform temperatures in the truck body than other types of refrigeration systems used. The cost of operating the truck bodies is no greater with the load in the truck than with it empty, hence it is common practice not to remove the return load where this system is used. The following operating costs for this system are given by the author. Depending upon power rates it costs from 15 cents to 50 cents per day with an average of 30 cents for a 500 to 600 gallon body. Daily depreciation amounts to 50 cents and service charge 10 cents per day or approximately 90 cents per day total cost.

It is stated that ice cream transport trucks sometimes used the above system of refrigeration but often resort to the constantly operating type, where a generator is mounted on the truck and driven by a power take-off from the transmission. Either direct expansion or partial hold-over coils are used with this system and it is claimed that best results are obtained where partial hold-over coils are provided. W.C.C.

**429. Refrigeration in the Dairy Industry.** R. A. BRODESSER, Southern Dairies, Inc., Washington, D. C. Ice Cream Field, *35:* 6, 12. June 1940.

Refrigeration is the most important factor to be considered in the food industry and the machinery required to produce it makes up the largest investment, it is claimed. Multi-stage compressors of the booster type and high speed refrigeration machinery are more efficient than the older types the author states.

It is claimed that a multi-process dairy plant, *i.e.,* one that produces ice cream, milk and other products needs three suction pressures, *viz.*—5, 20 and 30. Multiple header and booster systems make it possible to obtain any one of these pressures. The applications of each of these systems is then briefly discussed. The author refers to a system of refrigeration pipe arrangement as the "bin type hardening room," that his concern has used successfully. He claims that they prefer it to the low temperature blower system.

Referring to refrigerated trucks he claims that hold-over coils and dry ice are both giving satisfactory results.

Important steps in the development of ice cream cabinets are outlined. The trend has been towards a compact, light cabinet of sturdy construction, one which after installation will require the minimum of service. It is also claimed that in areas where the cost of dry ice equals that of purchased power, electrically operated compressors will be obsolete. W.C.C.

**430. Creating Gallonage.** A. W. SMITH, United Dairy, Springfield, Mass. Ice Cream Trade J., *36:* 5, 64. May 1940.

The United Dairy System, Inc., Springfield, Massachusetts, has hit upon a novel way for increasing the sale of ice cream to church socials, picnics,

weddings and parties. It will furnish a portable fountain without rental to any group sponsoring a gathering of any kind. W.H.M.

**431. The Use of Sugars in Ice Cream.** B. I. MASUROVSKY. Ice Cream Trade J., *36:* 5, 69. May 1940.

The author of this article has abstracted an original article entitled, "How One Sugar Compares with Another," by Dr. Stroud Jordan, Food Industries, volume 12, number 3, using sucrose as a standard and having a sweetening value of 100, other sugars have a sweetening value as follows: levulose—175; dextrose—66; corn syrup—30. The caloric value of sucrose is given as 1794 per pound, anhydrous dextrose, 1704 per pound, and hydrated dextrose, 1549 per pound. W.H.M.

**432. Consumer Educational Trends.** RACHAEL L. REED, Kansas City Dairy Council, Kansas City, Mo. Ice Cream Rev., *23:* 7, 39. Feb. 1940.

The ice cream industry stands to gain by cooperating with consumer groups. The reasons for a consumer movement and a brief history of it are given. It is suggested that ice cream manufacturers hold consumer leader conferences, arrange for plant visits, present industrial displays, etc. J.H.E.

**433. Efficient Personnel.—No. 1 Problem of the Retail Ice Cream Store Operator.** EDWARD THOM. Ice Cream Rev., *23:* 7, 30. Feb. 1940.

This is a portrayal of the policies and plan of operation of the 23 retail ice cream stores. The most important factor in successful operation is personnel. To train efficient managers and clerks and hold them is an everlasting job. An excellent means of building employee morale and bringing about a closer relationship between employer and employee has been a weekly news letter from the manager addressed to all supervisors, store managers, plant superintendents, and owners. J.H.E.

**434. Better Merchandising Through Drug Store Fountains.** M. A. NEWTON, Wendt's Cream Top Dairy, Niagara Falls, N. Y. Ice Cream Rev., *23:* 8, 35. March 1940.

Ice cream manufacturers should aggressively influence druggists to do a better job of merchandising ice cream. Old time fountain arrangements should be modernized. Too often the drug store fountain is unattended while the average store should have an attendant behind it at all times just as special retail ice cream stores have. J.H.E.

**435. A Method for the Accurate Sampling of Ice Cream.** A. C. MAACK AND P. H. TRACY, Univ. of Illinois, Urbana, Ill. Ice Cream Rev., *23:* 8, 36. March 1940.

There has been difficulty in getting a representative fat and solids test in fruit and nut ice cream due to the pieces of flavor material in the melted ice cream. Some testers merely strain out the added material and run the test on the remaining mix. This does not give accurate information even on the unflavored mix. It would be desirable to break up the material fine enough in order to get a representative sample.

An accurate method of sampling and testing fruit ice cream is described. The fruit in a four or five ounce sample of ice cream is thoroughly broken up by means of a malted milk mixer. Tests indicate an accurate test of the product can be made. Nut ice creams tend to show a considerable increase in fat content when the nuts are first broken up by the mixer. This is said to be due to the inclusion of the oil in the nuts. J.H.E.

**436. Sugars in Ice Cream.** R. J. TREBILCOCK, Corn Products Sales Co., New York. Ice Cream Rev., *23:* 8, 42. March 1940.

This is a review of the function of sugar in ice cream and a discussion of the various types of sugar. When dextrose is used to replace 25 per cent of the sucrose, the freezing point is lowered .65 of a degree F. J.H.E.

**437. Handling Seasonal Changes of Labor Requirements in an Ice Cream Plant.** I. R. KRILL, Moores and Ross, Inc., Columbus, O. Ice Cream Rev., *23:* 8, 38. March 1940.

A plan is described for handling the problem of seasonal employment in the ice cream industry. Meeting these requirements successfully involves three things: first, carefully selecting workers who have good health and are reliable; second, integrating the labor of the new employees with that of the old employees in such a way as to maintain efficient production and avoid waste; and third, managing by wise foresight and a few simple precautions to keep at a minimum the expense of unemployment compensation and industrial insurance.

The best market for summer labor is in two groups: first, women and girls who are seasonally employed in winter by department stores or seasonal industries; and second, students studying dairy manufacturing, who in the dull season of the ice cream business are attending school. J.H.E.

**438. Soliciting Consumers by Mail.** ANONYMOUS. Ice Cream Rev., *23:* 11, 23. June 1940.

A mail solicitation plan for reaching ice cream consumers is described. The experience thus far has been successful in gaining new customers.

J.H.E.

**439. Serum Solids for Ice Cream.** H. E. OTTING, M & R Dietetic Labs., Columbus, O. Ice Cream Rev., *23:* 9, 37. April 1940.

Several types of milk solids are available for ice cream. The kind used should depend on: (1) freedom from heated or cooked flavors; (2) stability or keeping quality; (3) laws of boards of health governing territory of distribution; (4) water absorbing ability; (5) composition of mix; (6) cost; (7) availability. The forewarming temperature, degree of concentration and characteristics of each type of serum solid source is discussed. J.H.E.

**440. What is Adequate Pasteurization of Ice Cream Mix.** T. V. ARMSTRONG, Ohio State Univ., Columbus, O. Ice Cream Rev., *23:* 9, 41. April 1940.

This article is a review of previous work on the thermal death points of pathogens in ice cream. The recent work on the *Escherichia-aerobacter* group of bacteria and the phosphatase test is considered. This leads the author to conclude that 150° F. for 30 minutes should be the minimum for adequate pasteurization. He also cites that the recommendations of the Committee on Ice Cream Sanitation of the International Association of Milk Sanitarians are 155° F. or higher for 30 minutes, or 180° F. or higher for 16 seconds as the short time method. J.H.E.

**441. How to Wash Ice Cream Equipment.** W. B. COMBS, Univ. of Minnesota, St. Paul, Minn. Ice Cream Rev., *23:* 9, 52. April 1940.

Equipment should first be rinsed with warm water and then dismantled. Prepare washing solution by adding washing powder to a pail of water and dissolve completely before use. Use a stiff brush to scrub all parts of equipment. Finally rinse with warm water and steam with a steam hose. The cleaning of special equipment is outlined. For metal equipment use to each 50 gallons of water a washing powder in the following amounts: 1 lb. neutral soda, $\frac{1}{2}$ lb. soda ash, and $\frac{1}{3}$ lb. trisodium phosphate. All equipment should dry quickly after cleaning. A chamois skin is recommended for polishing outside surfaces. J.H.E.

**442. How to Sterilize Equipment.** H. MACY, Univ. of Minnesota, St. Paul, Minn. Ice Cream Rev., *23:* 9, 54. April 1940.

This is an excellent article giving detailed instruction for sterilizing each piece of equipment commonly found in an ice cream plant. The system is rigid. In general, the equipment should be steamed until the condensate at the outlet of the equipment is above 180° F. for at least three minutes. Assembled equipment may be rinsed with chlorine solution just before use. This should contain 50–200 parts per million of available chlorine, depending upon the period of exposure. The author is of the opinion that a fresh water rinse is desirable following chlorine sterilization. J.H.E.

**443. New Competition for the Ice Cream Manufacturer.** H. H. SOMMER, Univ. of Wisconsin, Madison, Wis. Ice Cream Rev., *23:* 9, 94. April 1940.

Ice cream mixes and mix products for home use are on the market and are being tried by many house-wives. The type of products that have been placed on the market may be classified as follows: (1) mix bases consisting of stabilizer, sugar and flavors; (2) mix bases similar to number one but containing some skimmilk solids; (3) powdered ice cream mixes; (4) canned, sterilized ice cream mixes; (5) bottled, unsterilized, ice cream mixes. Some of these products involve difficulties with respect to mix composition, mix processing, freezing and whipping. Ice cream mixes, specially designed for home freezing and delivered freshly bottled on milk routes, at present are on the increase. Similarly, canned ice cream mixes have possibilities but also have some disadvantages, so the future of these products is difficult to predict. The belief is expressed that none of these products afford any real economy to the housekeeper but pride in her own handiwork may make for a permanent establishment of these home ice cream mixes. J.H.E.

**444. Reduce Power Costs.** ANONYMOUS. Ice Cream Rev., *23:* 10, 38. May 1940.

Operating in a community where purchased electric power cost 3¾ cents per kilowatt, a California ice cream manufacturer installed a new Diesel engine for generating his own current. After the experience of operating 8,000 hours, the owner concludes that his electricity now costs him less than one cent per kilowatt. J.H.E.

**445. Keeping down Bacteria Counts.** WESLEY SCHWEN, Schwen's Ice Cream Co., Blue Earth, Minn. Ice Cream Rev., *23:* 10, 40. May 1940.

This is a detailed explanation of how one ice cream plant practices good sanitation. Bacterial counts are run on all mixes and frequent line run tests and *E. coli* tests are used to determine source of contamination. Many helpful operating suggestions are given. J.H.E.

**446. Pan Condensed Ice Cream Mixes.** R. A. LARSON, Michigan State College, East Lansing, Mich. Ice Cream Rev., *23:* 10, 34. May 1940.

Baumé hydrometer readings were determined for a number of ice cream mixes made in the vacuum pan. For all the mixes studied within the range of 115–155° F. a 5° F. change in temperature caused a .2° Baumé change. Charts are given showing proper Baumé readings at various temperatures for 12 different mixes. The directions are given for predicting the correct Baumé reading of any composition mix.

**447. Proper Paint in the Ice Cream Factory.** J. W. THOMPSON, Pittsburgh Plate Glass Co., Milwaukee, Wis. Ice Cream Rev., *23:* 10, 54. May 1940.

This is an informative article giving much up-to-date information on the subject of proper paint for the walls, equipment and floors of dairy plants. Fungicide paints, fume resisting enamels, paint odor contamination, synthetic resins, the painting of refrigeration lines, and use of color and lighting are all discussed. J.H.E.

**448. Proper Accounting in the Ice Cream Business.** EDWIN STOVALL. Ice Cream Rev., *23:* 11, 33. June 1940.

This is a plea for simplification of accounting systems so that essentials are plainly and accurately portrayed. J.H.E.

**449. Some Points to Consider before Beginning Distribution of Frosted Foods.** RUSSEL BROWN, Birds Eye Frosted Foods Co. Ice Cream Rev., *23:* 11, 38. June 1940.

Ice cream manufacturers are said to handle 18½ per cent of all frosted food sold. This article is a discussion of a number of precautions to consider before venturing into the business. J.H.E.

**450. Point-of-Sale—Sanitation.** H. T. SMITH. Ice Cream Trade J. *36:* 6, 8. June 1940.

The use of paper cups and other single service containers have helped many ice cream dealers in solving the dishwashing problem and made it possible for them to comply with the stricter sanitary rules which are now in force in many cities. W.H.M.

**451. Kansas Ice Cream Survey.** H. E. DODGE. Ice Cream Trade J. *36:* 6, 36. June 1940.

The results of the annual ice cream surveys conducted by the Dairy Division of the Kansas State Board of Agriculture and the Dairy Department of the Kansas State College, show a steady improvement in the quality of the ice cream as indicated by the bacterial counts. The number of counter freezers increased from 62 in 1935 to 203 in 1938, followed by a decline to 202 in 1939. The average yearly gallonage was 3,000 gallons for the counter freezers and 28,000 gallons for the wholesale manufacturers. W.H.M.

**452. Early History of the Ice Cream Industry.** W. H. LIST, JR., Sec'y, Pa. and N. J. Assn. of Ice Cream Mfgs. Ice Cream Trade J., *36:* 5, 12. May 1940.

This article is very enlightening to those interested in the history of the ice cream industry. Pictures are presented and description given of the various developments which have taken place down through the years.

Starting with the introduction of the wholesale ice cream business in 1851 by Mr. Fussell in Baltimore, the story relates the experiences of many of the pioneers of the industry. Sanitation, source of supply, distribution, prices and regulations were problems which faced the ice cream manufacturers at the start of the twentieth century. Pasteurization, and homogenization of ice cream mix started about 1904. Federal pure food laws were passed in 1906 followed shortly thereafter by laws in several states. The brine freezer was introduced in 1902, the direct expansion in 1914 and the fore-runner of the present continuous freezer in 1928. Not until 1906 was electricity used as power for ice cream freezing. Uniform cost accounting was introduced about 15 years ago. Trucks were used for delivering of ice cream for the first time in 1907, and in 1925 the first mechanically refrigerated trucks were put into operation. Mechanically refrigerated ice cream cabinets were developed in 1920, and six years later dry ice was used as a refrigerant for ice cream. Paper ice cream cans made their appearance about 10 years ago followed by many types of paper ice cream containers. Ice cream associations and state universities have played an important role in the development of the ice cream industry. W.H.M.

**453. Gallonage.** VINCENT M. RABUFFO, Editor, Ice Cream Trade J. Ice Cream Trade J., *36:* 5, 16. May 1940.

The story of the development and operation of the Philadelphia Dairy Products Company, one of the nation's largest ice cream plants with an annual production of 5,000,000 gallons is described in this article. W.H.M.

**454. The Beginning of the Wholesale Ice Cream Business—1851.** M. T. FUSSELL. Ice Cream Trade J., *36:* 5, 37. May 1940.

This article relates the story of the beginning of the wholesale ice cream business by Jacob Fussell in Baltimore in 1851. The operation of the Baltimore plant was followed by one in Washington, D. C., 1856; Boston, 1862, and New York in 1864. W.H.M.

**455. Ice Box Competition.** HOWARD YAW. Ice Cream Trade J., *36:* 5, 28. May 1940.

Statistics are presented showing the marked increase in the sale of bottled carbonated beverages and fruit juices. The writer states that as the sale of these products for home consumption has increased there is evidence of a decline in the consumption of ice cream in the home. W.H.M.

**456. Homogenization, a Comparison of Pressure and Rotary Type Machines.** C. D. DAHLE, Pennsylvania State College, AND C. M. MOSS, Dairymen's League. Ice Cream Trade J., *36:* 6, 18. June 1940.

A gear type and eccentric type of rotary homogenizer for the homogeni-

zation of ice cream mix were compared with a pressure type. The author states that all machines gave satisfactory results from the standpoint of body, and texture of the ice cream, fat globule size overrun, and fat clumping. There was some difference in the rotary machines from the standpoint of pressure fluctuation temperature rise and sanitation and uniformity of operation. W.H.M.

## MILK

**457. High Quality Milk Production.** L. E. PARKIN. Pennsylvania State College Cir. 221, 12 pages. 1940.

This circular is for the producer of milk. It considers the essentials in the production of high quality milk involving the attendants, cows, environment, feed flavors, milking equipment and methods of milking and handling the milk. W.E.P.

**458. Some Legislative Aspects of Chocolate Milk Distribution.** GIDEON HADARY. Milk Dealer, *29:* 8, 78–82. May 1940.

A brief discussion of the laws and regulations governing the production and sale of chocolate milk. In summarizing his discussion the author states that one can regard chocolate milk primarily as: (1) an outlet for the sale of whole milk, overlooking the fact that it is a beverage; (2) a beverage, overlooking the fact that it contains milk; (3) a beverage containing milk.

The first two approaches are wrong. The first approach is that taken by the courts in Florida, while the second is taken by the State of Kentucky. The third approach, the one that is the best, takes into consideration that the drink is an outlet for milk sales; yet, the fact that it is a beverage superior to others by the presence of milk in it is not overlooked. Wise municipal regulation will set this approach as criteria in establishing chocolate milk legislation. C.J.B.

**459. What We Know about Homogenized Milk.** F. J. DOAN, Pennsylvania State Collgee, State College, Pa. Milk Dealer, *29:* 8, 42–52. May 1940.

The advantages, disadvantages, properties and characteristics and the problems in the production and distribution of homogenized milk are discussed. It is also pointed out that where only a medium efficiency of homogenization is required, the rotary homogenizer will give as good results as the piston; but if high efficiency of homogenization is needed, then the piston machine will usually give superior results. C.J.B.

**460. Control of Flavor in Milk Heated to High Temperature.** I. A. GOULD, Dept. of Dairying, Michigan State College, East Lansing, Mich. Milk Dealer, *29:* 8, 70–76. May 1940.

Report of a study to determine the possibility of using a combination of

both copper and homogenization on high-temperature treated milk to control the cooked and oxidized flavor. The author summarizes his work as follows:

Milk heated to 180° F. and then treated with small quantities of copper salts will lose its cooked flavor and become oxidized. However, if the milk is homogenized either before or after the addition of copper, the oxidation is prevented and the milk in time will assume a normal flavor.

Milk to which was added 1.5 to 2 p.p.m. of copper and which was then homogenized, showed practically no cooked flavor after 24 hours of storage, and the milk was fine-flavored even after 120 hours. When less copper was used, the cooked flavor disappeared somewhat more slowly. The cooked flavor disappeared slightly more rapidly if the copper was added at 145° F. than when added at 180° F. Somewhat similar results were secured when the copper was added after homogenization.

The data presented herein offer a practical application of previous findings dealing with the cooked flavor. The results show that it is possible to secure an excellent, normal-flavored milk even though the milk has been subjected to temperatures sufficiently high to cause a strong cooked flavor to appear. The combination of copper salts with homogenization in a proper manner might be used under certain conditions to control milk flavors of highly heated milk.

Proper use of the findings of this paper would permit an operator to prepare a milk with low curd tension, as brought about by heat and homogenization without the disagreeable off-flavor which such milk usually possesses. It is realized, however, that application of these findings in a commercial manner must be done with the approval of health officials.

C.J.B.

## PHYSIOLOGY

**461. Destruction of Ascorbic Acid in the Rumen of the Dairy Cow.** C. A. Knight, R. A. Dutcher, N. B. Guerrant and S. I. Bechdel, Departments of Agricultural and Biological Chemistry and Dairy Husbandry, Pennsylvania State College. Proc. Soc. Exp. Biol. and Med., *44:* 90, 1940.

Neither the feeding of 100 grams and 150 grams of synthetic ascorbic acid mixed with corn silage nor the placing of 100 grams of ascorbic acid directly in the rumen through a fistula opening increased the ascorbic acid values of the blood plasma and of the milk when compared with those values obtained while the cow was on a standard ration unsupplemented with the vitamin. A slight increase was noticed in the amount of ascorbic acid found in the 24-hour sample of urine for the periods during which the vitamin was administered. A rapid and pronounced destruction of ascorbic acid in

the rumen was demonstrated by removal and analysis of samples of the rumen contents at regular intervals after the cow had been fed. Ascorbic acid added to rumen contents in vitro and stored in a dark-glass, stoppered bottle at 39°–42° C. disappeared at much the same rate as that of the in vivo experiments. R.P.R.

**462. The Effect of Certain Experimental Conditions upon the Thyrotropic Hormone Content of the Albino Rat.** C. W. TURNER AND P. T. CUPPS, Department of Dairy Husbandry, University of Missouri. Endocrinology, *26:* 1042, 1940.

A study of the thyrotropic hormone content of the pituitaries of albino rats of both sexes, weighing between 150 and 200 gm., following various periods of gonadectomy was reported. Following gonadectomy, the thyrotropin content of the A P of both males and females was reduced slightly after 20 days and rather markedly after 66 days. Replacement therapy with estrogen at the rate of 40 r. u. and androgen at the rate of 200 gamma daily appeared to maintain the normal level of thyrotropin in the castrate female but not in the male. Thyroidectomy of male rats for periods of 40 days and 6 months resulted in a reduction of about 50 per cent in the thyrotropin content of the A P. In contrast, females similarly treated maintained their normal content or showed a slightly increased level of hormone. R.P.R.

**463. The Comparative Assay of Gonadotropic Substances on Rats, Mice and Chicks.** JOHN S. EVANS, LEONARD HINES, ROGER VARNEY AND F. C. KOCH, Dept. of Biochemistry, University of Chicago. Endocrinology, *26:* 1005, 1940.

In the assay of unfractionated pituitary extracts, the mouse uterus was about 66 times as sensitive as the rat ovary, and about 10 times as sensitive as the chick testes. In the assay of pregnant mare serum preparation (gonadogen), the mouse uterus was about 60 times as sensitive as the rat ovary, and about 90 times as sensitive as the chick testes. In the assay of normal male urine preparation (prospermin), the mouse uterus was about 90 times as sensitive as the rat ovary, and about 55 times as sensitive as the rat uterus. The response of the chick testes to normal male urine was doubtful. The mouse uterus was about 30 times as sensitive as the rat ovary to menopause urine preparation (gamone) and about 6 times as sensitive as the rat uterus. The response of the chick testes to menopause urine was doubtful. R.P.R.

**464. Utilization of the Ketone Bodies in Normal Animals and in Those With Ketosis.** RICHARD H. BARNES, D. R. DRURY, P. O. GREELEY AND A. N. WICK. Department of Physiology, School of Medicine,

University of Southern California, and Scripps Metabolic Clinic. Amer. Jour. Physiol. *130:* 144–150. 1940.

The authors suggest that the production of ketone bodies by the liver and utilization of them by the other tissues is an important, though not necessarily inevitable, route for the catabolism of fatty acids. When the organism is in a state of ketosis, increases in metabolic rate (as in exercise) probably increases the rates of production and utilization of these substances.

The results indicate that from 30 to 80 per cent of the energy requirements of the tissues (rabbits, goats, dogs) in ketogenic states may be supplied by combustion of ketone bodies.

The authors believe that more than one molecule, possibly four, of ketone bodies may be formed per molecule of fatty acid catabolized. D.E.

**465. Induction of Lactation in Goats with Diethylstilboestrol Dipropionate.** S. J. Folley, Helen M. Scott Watson and A. C. Bottomley. National Institute for Research in Dairying, Reading. Proc. Physiol. Soc., Jour. Physiol. *98:* 15–16. 1940.

One gram of one per cent diethylstilboestrol dipropionate ointment was applied three times a week to the udders of three virgin female goats and daily milking begun. After a latent period of 30 days during which a few ml. of fluid were secreted daily, there was a sudden increase in milk yield to a maximum of 1500 ml. daily and then a slow decline. The milk secreted was normal and the milk production curve resembled a normal lactation curve. The results with a virgin heifer were disappointing, the secretion never passing the colostral stage. These experiments indicate that oestrogens may not inhibit lactation in ruminants but, at least when injected into goats in limited amounts, will cause udder development and copious secretion of normal milk without need for prolactin treatment. D.E.

**466. Glycogen and Calcification.** G. E. Glock. Dept. of Physiology, Bedford College, University of London. Jour. Physiol. *98:* 1–11. 1940.

It is suggested that in both tooth and bone development, the glycogen of the bones might first initiate the differentiation and later serve as a primary source of the phosphoric esters required for calcification. If this is true the inhibitory effect of NaF on bone calcification might be attributed to the low glycogen content which resulted from the administration of flourine.

D.E.

**467. The Partition of Serum Calcium about the Time of Parturition in the Dairy Cow.** J. Duckworth and W. Godden. The Rowett Institute, Aberdeen, Scotland. J. Dairy Res. *11:* 9–14. 1940.

Data are given of the variations in the calcium ion, ultrafiltrable calcium

complex, non-ultrafiltrable calcium complex and protein-bound calcium at normal parturition in the dairy cow. All the values found for total serum calcium were higher a few days before calving than those found either a few weeks before or after calving. At the time of actual calving, however, there was generally a reduction of about 10 per cent in the total calcium.

The values obtained for calcium ion ($Ca^{++}$) were generally, but not always, maximal at or near the time of calving.

The values for the ultrafiltrable calcium complex and for non-ultrafiltrable calcium complex all showed a reduction at the time of calving.

The values for protein-bound calcium were generally, but not invariably, increased near the time of calving. S.T.C.

**468. A Long-Term Study of the Partition of Serum Calcium in Ayrshire Cows.** W. GODDEN AND J. DUCKWORTH. The Rowett Institute, Aberdeen, Scotland. J. Dairy Res. *11:* 15–21. 1940.

The following average fractionation of serum calcium in the dairy cow were reported:

| | |
|---|---|
| Calcium ion ($Ca^{++}$) | 10–12 per cent. |
| Ultrafiltrable calcium complex | 40–45 per cent. |
| Non-ultrafiltrable calcium complex | about 20 per cent. |
| Protein-bound calcium | about 25 per cent. |
| Ultrafiltrable calcium | 50–53 per cent. |

S.T.C.

## MISCELLANEOUS

**469. Cold Storage Locker Operation.** ANONYMOUS. Ice Cream Rev., *23:* 11, 40. June 1940.

This is a brief report of the second annual cold storage locker operators' conference at the University of Wisconsin, April 30 to May 1, 1940. Suggestions for handling frozen meat products are given. J.H.E.

**470. What Shall I Use for Fuel in the Dairy Plant.** S. KONZO, Eng. Exp. Sta., Univ. of Illinois, Urbana, Ill. The Dairy World, *19:* 2, 16. July 1940.

The author briefly summarizes the advantages of coal (hand and stoker fired), oil and gas as boiler fuels on a comparative basis. He gives the costs of the three types of fuel, under stated conditions, as: 3.8 cents for coal, 4.9 cents for oil and 7.5 cents for gas, per 100,000 B.T.U. From the data presented costs under other price conditions can be readily calculated. Methods by which maximum combustion efficiency may be obtained are presented. F.J.D.

**471. Making Figures Effective to Management.** RAY C. PERKINS, Adohr Milk Farms, Los Angeles, Calif. The Assn. Bull. Intern. Assn. Milk Dealers. 32nd year. No. 15: 341–346. March 1940.

To be effective, reports should be brief, concise, definite and imaginative. The results of all branch plants should be included on each report for comparative purposes. Present to management in permanent typed form only the reports that carry an effective message. Seven such reports are described. E.F.G.

**472. Problems of Internal Audit Control.** PAUL L. SCOTT, Bordens Delivery Co., San Francisco, Calif. The Assn. Bull. Intern. Assn. Milk Dealers. 32nd year. No. 15: 347–352. March 1940.

One purpose of internal audit control is to remove the temptation for the employee stealing property or money by involving at least three persons in a suitable system of control. The essential details of such a system are outlined. An effective plan of internal control should cover everyone from routeman to president and be sufficiently rigid so that each one will recognize the futility of any criminal "intention" in handling company transactions. An extensive list of references in connection with internal control operation and internal auditing is appended. E.F.G.

**473. Approaches to Budgetary Control through Planned Performance.** ANSON HERRICK, C.P.A. The Assn. Bull. Intern. Assn. Milk Dealers. 32nd year. No. 15: 353–362. March 1940.

The characteristics of budgets in industry for different purposes and different operations are given. It is also explained how these budgets may be used to control costs and operations. In situations where production is not uniform the unit cost basis must be used. It is believed that standard cost rates are adaptable in the majority of dairy manufacturing processes and are at least the most convenient and economical basis for using planned performance and costs as efficiency standards for the controlling of actual production costs. E.F.G.

**474. Is Collective Bargaining the Answer to our Labor Relations Problems?** ALMON E. ROTH, President, San Francisco Employers' Council. The Assn. Bull. Intern. Assn. of Milk Dealers. 32nd year. No. 16: 367–382. May 1940.

Strikes and threats of strikes have far reaching economic consequences in redistribution of markets. Many concrete instances are given. The author addresses himself to the question: What are the chances of establishing peaceful labor relations through collective bargaining? The many essential factors in orderly collective bargaining are discussed together with unsolved difficulties. Conceding a conflict of interests between employer

and employee the author states that the most that can be hoped for is to develop a fair and honest attitude on the part of each of the parties toward the other and to insist that peaceful means be developed for settling disputes without costly interruptions or unnecessary hardships. E.F.G.

**475. Prevention of Fly Contamination on the Farm.** L. J. HOIS, Geuder, Paeschke & Frey Co., Milwaukee, Wis. Milk Dealer, *29:* 8, 66–68. May 1940.

The use of strainer covers and screened racks for milk pails and cans is recommended as a means of fly control. This localized control is in addition to the usual methods of fly control such as destroying or treating feeding and breeding places. C.J.B.

**476. Tackling the Distribution Problems of the Dairy Industry.** L. R. SCAFE, White Motor Co., Cleveland, Ohio. Milk Dealer, *29:* 8, pp. 34–35, 82–84. May 1940.

The distribution problems of the dairy industry are discussed mainly from the transportation standpoint. The author states that: "In the field of distribution lies our greatest future opportunity of cutting costs and increasing profits." C.J.B.

**477. Proper Paint in the Dairy Plant.** J. W. THOMSON, Pittsburgh Plate Glass Co., Milwaukee, Wis. Milk Dealer, *29:* 8, pp. 32–33, 61–64. May 1940.

A discussion of the proper paint to use in different sections of a dairy plant. C.J.B.

**478. Water Supply vs. Quality in the Dairy and Ice Cream Plant.** M. E. PARKER, Beatrice Creamery, Chicago, Ill. Ice Cream Trade J., *36:* 5, 31. May 1940.

The importance of the water supply in the production of quality dairy products is discussed. Since water is an ingredient used in the manufacture of sherbets, ices, fruit juice drinks and other dairy products, it should be free from sediment and objectional odors which might be imparted to the finished product. By tasting and smelling water which has been heated to 100° to 120° F. the operater can usually detect obvious defects.

The chemical character of the water used for cleaning purposes may be responsible for milk stone accumulation on equipment unless the proper cleaner is selected.

Metallic and other off flavors have occurred in dairy products contaminated with water or steam which contained impurities. The use of steam separators to remove impurities and the chlorination of wash water have been effective measures in preventing off flavor from infected water.

W.H.M.

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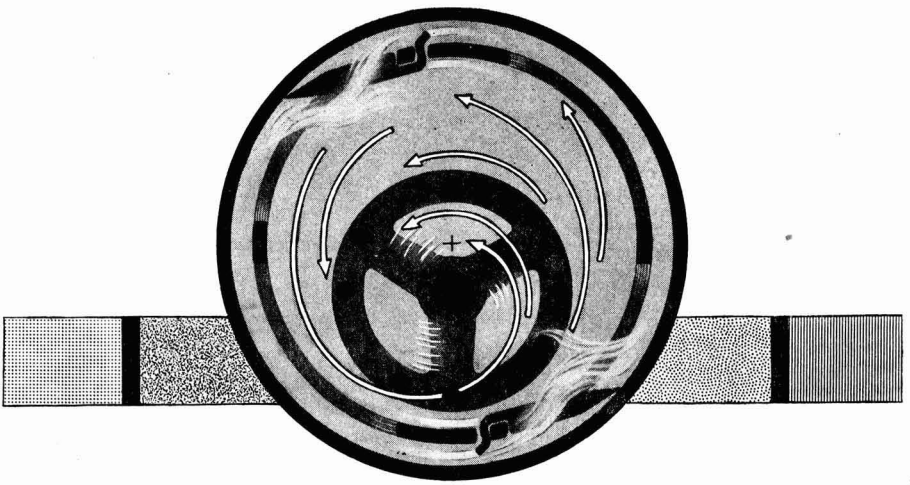

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