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Contents

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JOURNAL OF DAIRY SCIENCE

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THE INFLUENCE OF ESTRUS ON WEIGHT OF DAIRY HEIFERS[1]

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One of the principal objections to the use of heifers as experimental animals in pasture research is the disturbance caused by estrus or heat periods. Little or no data are available in the literature regarding the magnitude of weight differences caused by this phenomenon. Such information is especially important when 1-day weights are used.

EXPERIMENTAL DATA

Dairy heifers were used in a pasture experiment conducted at the Kentucky Agricultural Experiment Station from 1936 to 1948, inclusive. Holstein, Jersey and Swiss-Jersey cross-bred heifers were involved. These heifers were weighed on 3 consecutive days at 2-wk. intervals while on the experimental pastures. While on pasture, they received no supplementary feed except salt and bone meal. In addition, all other heifers in the herd were weighed on 3 consecutive days at monthly intervals. Heifers, when not on the pasture experiment, received mostly pasture during the summer and hay and some grain during the winter.

Heat periods occurring on weigh days were recorded. In 241 instances of 4,192 3-day weight series, heifers were in heat on one of the weigh days. Because some heifers were in heat at more than one series of weighings, 203 different heifers were involved. These were classified in groups according to the weigh-day on which they were in heat (table 1).

RESULTS

A large proportion of the heifers weighed less on the day in heat than on the days preceding or following the day of heat. Table 2 shows the per cent affected and the range in weight variations.

The age of the heifers in this study varied from less than 1 yr. to more than 2 yr., and their weight ranged from 300 to 1,200 lb., averaging about 628 lb. on the day in heat. The average weight of the Holsteins was 789 and the Jerseys 486 lb. The average weight depression was approximately 3 per cent of the weight on the day of estrus.

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[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

The larger heifers tended to lose more weight when in heat than the smaller heifers. The coefficient of correlation between the weight of the heifers and the drop in weight on the day in heat was 0.42. This figure is highly significant.

TABLE 1

Number of instances and number of heifers in heat on day when weighed

	No. of instances			
	Jersey	Holstein	Swiss-Jersey	Total
Group A—in heat 3rd weigh day	41	38	1	80
Group B—in heat 2nd weigh day	45	42	4	91
Group C—in heat 1st weigh day	40	28	2	70
Total	126	108	7	241
	No. of heifers			
Group A—in heat 3rd weigh day	38	30	1	69
Group B—in heat 2nd weigh day	39	30	3	72
Group C—in heat 1st weigh day	36	24	2	62
Total	113	84	6	203

DISCUSSION

The occurrence of estrus apparently caused marked changes in the physiological routine of dairy heifers. In a large majority of cases their weight was lowered during this period. Presumably this decrease in weight resulted from failure of the heifers to eat and/or drink normally and to some extent from their taking an unusual amount of exercise.

Considerable variation in effect of estrus on weights of heifers was found. This probably was caused by one or more of several factors such as size of heifer, amount of fill, length of time the heifer had been in heat when weighed and difference in magnitude of physiological effect of estrus in individuals.

The highly significant correlation between the weight of the heifer on the

TABLE 2

Effect of estrus on weight of dairy heifers

	2 d. before heat	Day before heat	Day after heat	2 d. after heat
	(%)	(%)	(%)	(%)
Weighed more than on day in heat	86.3	93.6	83.8	92.9
" less " " " " "	11.2	5.8	13.7	7.1
" same as " " " "	2.5	0.6	2.5	0.0
	lb.	lb.	lb.	lb.
Range in weight variations	−18 to +66	−20 to +66	−26 to +70	−18 to + 58
Av. difference in weight	19.3**	19.5**	16.8**	17.8**
Standard error of estimate	±1.96	±1.06	±1.16	±1.76

** Highly significant, $P < 0.01$.

day in heat and the change in weight from the day before indicates a close relationship to size. The amount of fill or contents of the alimentary tract should be roughly proportional to the size of the animal.

The heifers were weighed at about 8 : 00 a. m. Some of them may have been in heat since the previous afternoon and others only a few hours. The exact time of onset of estrus was not known but the length of this interval probably affected the loss in weight. Since some heifers show more marked effect of disturbance than others, it is reasonable to suppose that the intensity of the effect would result in variations in the amount of weight lost during heat periods.

No great difference was noted between Holstein and Jersey heifers when the per cent change in weight is considered.

The occurrence of estrus interrupted normal gain in weight slightly. Normal daily gain for a mixed group of Holstein and Jersey heifers of about 600 lb. should approximate 1 lb. This was the case with Group A which was in heat on the third weigh day. However, weights for Group B, in heat on the middle weigh day, indicate that full recovery was not made on the day following estrus.

When single weigh days are used with dairy heifers as experimental animals, the occurrence of estrus may constitute a considerable error. If a normal daily gain of 1 lb. was expected, the temporary weight loss due to estrus might cancel out expected gain for a 2- to 4-wk. period, depending on the size of the heifer. It is suggested that when single weigh days are used with dairy heifers that if they are in heat on weigh days, 3 per cent of their recorded weight be added in order to correct for the effect of estrus.

SUMMARY

The effect of estrus on weight of dairy heifers in 241 instances among 203 heifers is reported.

Loss in weight on day of estrus as compared with the previous day was approximately 3 per cent of the weight on the day of estrus.

Correlation between pounds lost and size of heifer was highly significant.

It is suggested that when 1-day weights are used with dairy heifers as experimental animals, 3 per cent of their weight should be added as a correction factor if they are in heat on weigh day.

The occurrence of estrus in dairy heifers results in a loss in weight and when single-day weights are used may be corrected by the addition of 3 per cent of their actual weight on the day in heat.

REPEATABILITY OF THE ESTROUS CYCLE LENGTH IN DAIRY CATTLE[1]

DURWARD OLDS AND D. M. SEATH[2]

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Much has been published concerning the estrous cycle of dairy cattle. Asdell (2) and Roark (13) have given excellent reviews of literature on the subject. McNutt (11) observed 19 animals and found the average cycle to be 21 days. Hammond (6) states the length of the normal estrous cycle in the cow averages 19.5 days but varies from 17.5 to 24 days. Werner *et al.* (17) observed the estrous cycles of 35 dairy heifers and found that 65.9 per cent of 82 cycles ranged between 18 and 24 days. Alba (1) reported the modal cycle length as 20 days for unbred heifers and 21 days for cows. He found that 85 per cent of the cycles in heifers fell between 18 and 22 days, whereas in cows 84 per cent fell between 18 and 24 days. The mean for heifers was found to be 20.23 days with a standard deviation of 2.33 days. For cows the mean was 21.28 days with a standard deviation of 3.68 days. Lasley and Bogart (8) found the mean cycle length for beef cattle to be 19.6 $\pm$ 0.12 days, the modal length being 20 days, and 79 per cent falling between 17 and 23 days. Roark (13) recorded the length of 504 estrous cycles of 110 cows and found that about 80 per cent of the cycles fell between 18 and 24 days. The mean cycle length was 21.4 days. Chapman and Casida (4) found that intervals between heats varied from 2 to 200 days. The mode was 21 days both for cows which were bred at the previous heat and for those which were not bred. However, the means of the two groups differed. The non-copulatory cycles averaged 32 days while the copulatory cycles averaged 37 days. When all cycles over 33 days were excluded, the mean for the non-copulatory group was 21 days and for the copulatory group it was 22 days, the differences being significant.

In checking herds experiencing breeding problems, the question arises as to whether cows having estrous cycles of the usually expected 18 to 24 days show a higher breeding efficiency than others. Van Demark and Moeller (16) found that cows bred following a cycle of 2 to 17 days had a significantly lower breeding efficiency than cows bred following longer cycles. The breeding efficiency of cows following cycles of 18 to 25, 26 to 35, 36 to 50 and 51 to 72 days was not significantly different from the mean.

Another question is whether cows show an individual tendency to have normal cycles, or similarly, whether they have abnormal cycles and show a tendency to continue them. Asdell *et al.* (3) found evidence that individuality of the cow or heifer is the principal cause of variation in cycle length. It also was stated that

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2 The authors wish to acknowledge the liberal assistance of Levi Oliver, a graduate assistant, in tabulating the data used in this study.

the strong tendency for cows with abnormally long cycles to repeat was the cause of most of the variation in individuals. Chapman and Casida (5) applied analysis of variance to all cycles and to cycles limited to a range of 4 to 33 days; they found no significantly inherent periodicity of estrus. However, when 17- to 27-day cycles were analyzed, each animal had a very definite individuality in cycle length. They stated that 41 per cent of the observed variance would disappear if all the estrous cycles were those of one individual.

It may be seen in the above review that many investigators have reported the mean cycle length as approximately 21 days, while others have reported a mean length of over 30 days. The reason for this discrepancy appears to be that some of the workers omitted excessively long intervals before calculating the mean. However, it is possible that some of the workers were able to observe the comparatively small number of cows closely enough to eliminate long intervals due to missed heat periods. Olds (12) found among 5,304 cows returning for second service in routine artificial breeding, that 34.1 per cent returned in 37 days or more. It would be of value to know to what extent these long intervals are due to "missed" heat periods.

With an increased effort being made to evaluate the importance of early fetal or embryonic death followed by unseen abortion or reabsorption (15), the following study was undertaken to bring together much of the information concerning the estrous cycle and to supplement those phases of the knowledge which are as yet rather inconclusive.

EXPERIMENTAL PROCEDURE

Breeding records for the Kentucky Agricultural Experiment Station dairy herd were studied covering a period of 18 yr. (1928–1946). Estrous cycle lengths, as indicated by length of time observed between heat periods, were tabulated for 278 cows of the Holstein and Jersey breeds and included 3,776 intervals or an average of 13.6 intervals per cow. The possibility of fallacy in the records is, of course, admitted. It must be recognized that some cows probably were missed or were not recorded as being in heat. Likewise, it is possible that early abortions occurred unnoticed or fetal death and resorption occurred. However, starting in 1928, a very satisfactory method of record keeping was adopted and so far as can be determined by studying the records, as well as conferring with staff members who were present during that period, the herdsman made very close observations and recorded them carefully. All cows and heifers were checked for heat periods at least twice a day and nearly always at a time when they were free to move about amongst other cows. Therefore, the authors believe these records will portray what many practical dairymen should expect.

In tabulating, a distinction was made between intervals following service and those not following service. Breeding efficiency, based on percentage of services resulting in conception also was determined for cows having had cycle lengths of different numbers of days.

Repeatability of estrous cycle length was determined by comparing the values for various sources of variance in line with the intraclass correlation technique as outlined by Snedecor (14).

RESULTS

The mean length of 3,776 estrous cycles for 278 cows was 32.4 days, with a range from 1 to 549 days. It is probable that the short intervals of 1 or 2 days were continuous heat; however, since there were only six such intervals it is believed they will not noticeably bias the results in this study. As may be seen in figure 1, there were many intervals well over 30 days. About 11.6 per cent of the intervals were 53 days or over. No cases of known pregnancy or observed abortion were included in these long intervals. However, as previously stated, it is possible that some heat periods were missed or not recorded. There may have been unseen abortions or embryonic death and resorption. The modal length was 22 days and this particular length of cycle represented about 12 per cent of the intervals. It seems reasonable that the modal length (22 days) should repre-

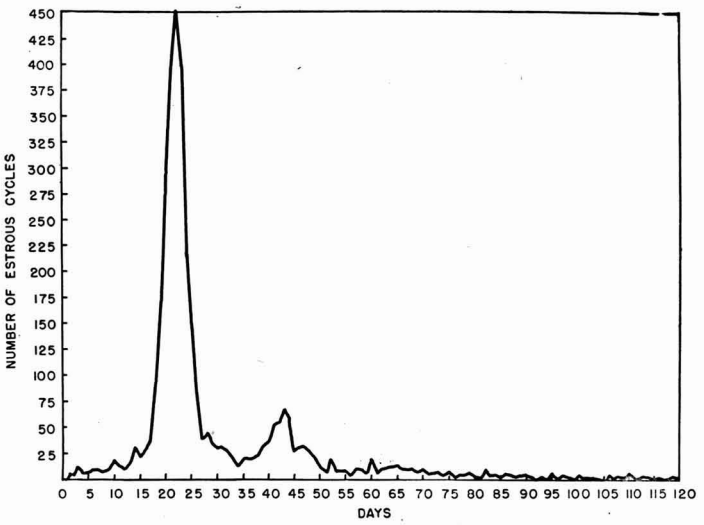

FIG. 1. Frequency of estrous cycles having lengths of 1 to 120 days.

sent the center point for so-called "normal" intervals. In order to get a mean of approximately 22 days, it was necessary to eliminate all intervals of 36 days or over. The standard deviation, after eliminating these long intervals, was 4.6 days. In this case 82.8 per cent of the intervals fell between 17 and 26 days.

Estrous cycle lengths following heat periods at which the cows were bred averaged 35.7 days, whereas, following heat periods at which the cows were not bred, the cycles averaged 30.6 days. The difference between these means was found to be highly significant ($P < 0.01$). As may be seen in table 1, the percentage of intervals in the 17- to 26-day group was 15.6 percentage units higher when the cows had not been bred at the previous heat. The percentage of intervals in the 27- to 33-day and 53-day or over groups for the cows not bred at the previous heat were 8.2 and 5.0 percentage units lower, respectively, than for those in the group following heat periods at which the cows were bred. Statis-

tically, these differences were highly significant. It should be explained that the 17- to 26-day interval was established by extending the mean a distance of one standard deviation on either side. This mean (21.75 days) was purposely made to approximately equal the modal cycle length (22 days) by eliminating cycles

TABLE 1

The time at which estrus recurred in cows following service and those not following service

No. of days	Percentage of 1,347 intervals following service	Percentage of 2,429 intervals not following service
1–16	4.1	6.5
17–26	51.1	66.7
27–33	11.5	3.3
34–52	18.5	13.7
53 or more	14.8	9.8
	100.0	100.0

of 36 days or more. The 34- to 52-day interval was intended to include all intervals which represented approximately two "normal" cycles. The differences between intervals following service and those not following service (table 1) would indicate that about 18 per cent of the cows returning to heat after 27 days or more had a prolonged cycle which was probably directly attributable to service. These abnormally long cycles may have been due to early fetal or embryonic deaths or to cases of endometritis, initiated or aggravated by service, which resulted in persistence of the corpus luteum. There may be other possible explanations. Chapman and Casida (5) stated that potentially fertile matings are followed by longer cycles than are services with a vasectomized male or no mating at all, and Marion *et al.* (10) found that sterile copulation had no effect on the length of the subsequent estrual cycle.

TABLE 2

Analysis of variance relative to the estrous cycle length in dairy cattle (includes all cycles)

Source of variance	Degrees of freedom	Mean square	Composition of mean square
Total	3,775		
Between cows	277	1,218.2**	E + cC
Within cows	3,498	608.9	E

c = average number of estrous cycles per cow, or 13.6*

$$C = \text{variance due to cow} = \frac{(E + cC) - E}{c} = 44.8$$

$$\text{Repeatability} = \frac{C}{E + C} = 0.069$$

** P = < 0.01.

* This mean was calculated by the procedure outlined by Hetzer *et al.* (7).

As may be seen in table 2, the analysis of variance when applied to the data showed that there was significantly greater variation in cycle length between cows than there was within cows. The composition of the mean square indicated a repeatability for single records of 0.069, or 6.9 per cent, for length of estrous

cycle. If one considers the average of 2, 3 or 4 estrous cycles, the estimated variance (9) accounted for would be 13, 18, and 23 per cent, respectively, instead of the 6.9 per cent for one record only.

Since there was some uncertainty concerning the number of long intervals which in reality represented double or triple cycles, it was decided to determine the effect of omitting all intervals of 36 days or more by applying the analysis of variance to the remaining data. The results of this analysis (table 3) showed

TABLE 3

Analysis of variance relative to the estrous cycle length in dairy cattle after all intervals of 36 days or over have been omitted

Source of variance	D/f	Mean square	Composition of mean sq.
Total	2,786		
Between cows	274	37.21**	$E + cC$
Within cows	2,512	19.66	E

$c = 10.13$

$C = \frac{37.21 - 19.66}{10.13} = 1.73$

$\text{Repeatability} = \frac{C}{E + C} = .081$

** $P = < 0.01$.

that the variance between cows was still significantly greater than that within cows. However, the variance due to cow was about 1/25th as great as when all intervals were considered. The repeatability was 0.081, which was only slightly higher than when all intervals were considered.

TABLE 4

The effect of estrous cycle length on fertility of dairy cattle

Length of previous estrous cycle	No. of cows bred	Per cent conceived
(*d.*)		
1–16	58	29.3
17–26	1,097	39.8
27–33	148	24.3
34–52	322	44.4
53 or more	239	39.3
	1,864	39.0

Chi-square = 19.6 $P = < 0.01$

Breeding efficiency data were available for 1,864 cows which were bred following estrous cycles of varying lengths. The results are shown in table 4. It was found that cows bred following a cycle length of 1 to 16 or 27 to 33 days had a significantly lower breeding efficiency than cows bred following a cycle of approximately 3 wk., 6 wk. or longer. Breeding efficiency also was determined for cows bred following each of the cycle lengths from 17 through 26 days. The differences here were not significant.

SUMMARY

Breeding records for the Kentucky Agricultural Experiment Station dairy herd were studied covering a period of 18 yr. (1928–1946). Estrous cycle lengths were tabulated for 278 cows and included 3,776 intervals or an average of 13.6 intervals per cow. The mean cycle length was 32.4 days with a range from 1 to 549 days. About 11.6 per cent of the intervals were 53 days or over. There were no cases of known pregnancy or observed abortion included in these long intervals. The modal length was 22 days and this cycle length represented about 12 per cent of the intervals. In order to get a mean of approximately 22 days it was necessary to omit all intervals of 36 days or more. The standard deviation, after eliminating these long intervals, was 4.6 days. In this case 82.8 per cent of the intervals fell between 17 and 26 days.

Estrous cycle lengths following heat periods at which the cows were bred averaged 35.7 days, whereas following heat periods at which the cows were not bred, the cycles averaged 30.6 days. The difference between these means was highly significant.

Among cows which were not bred at the previous heat, the percentage of cycles in the 17- to 26-day or "normal" group was 15.6 percentage units higher than for cows which were bred. The percentage of intervals in the 27- to 33-day and 53-day or over group for the cows not bred at the previous heat was 8.2 and 5.0 percentage units lower, respectively, than for those in the group following service. These differences also were statistically highly significant.

The analysis of variance, when applied to the data, showed that there was significantly greater variation in cycle length between cows than there was within cows. The composition of the mean square indicated a repeatability for single records of 0.069, or 6.9 per cent for length of estrous cycle.

When all intervals of 36 days or over were omitted, the variance due to cow was about 1/25th as great as when all intervals were considered. The repeatability was still only 0.081.

The breeding efficiency of cows following cycles of approximately 3 wk., 6 wk. or longer was significantly higher than for cows bred following cycles of 1 to 16 or 27 to 33 days.

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THE KEEPING QUALITY OF WHOLE MILK POWDER. III. THE USE OF ANTIOXIDANTS—ASCORBIC ACID AND SODIUM CITRATE[1, 2]

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The part played by ascorbic acid in the prevention of oxidized flavor in whole milk powder needs clarification. Sodium citrate has been reported (8) as having antioxidant properties and also is useful in adjusting the salt balance.

REVIEW OF LITERATURE

Hollender and Tracy (8) found ascorbic acid and sodium citrate to have slight antioxidant effect when used in whole milk powders at the levels of 0.01 and 0.2 per cent, respectively, on a reconstituted basis. Findlay *et al.* (4) tested a number of substances for their antioxidant activity in laboratory-made spray-dried whole milk powders. Ethyl gallate and ascorbic acid proved most promising, materially increasing the storage life of the powders without adding any foreign flavor. Ascorbic acid was used in concentrations of 0.008 to 0.306 per cent (10 to 382 mg. per liter on a reconstituted basis) and storage life increased with increasing quantities of ascorbic acid used in the powder, but not in direct proportion to the amounts used.

Findlay *et al.* (4) found that 0.44 per cent ascorbic acid in whole milk powders (about 500 mg. per liter on a reconstituted basis) retarded development of tallowiness in low-moisture powders but had no appreciable influence on non-fatty deterioration in powders of high moisture content (4.3 per cent or higher).

The deterioration rate of ascorbic acid in powders during spray drying and storage has been given by several workers. Wright and Greenbank (15) found with single gas-packed samples, that the control powder without added ascorbic acid decreased in apparent ascorbic acid content in storage at 37° C. during the first 4 mo. Then it gradually gained in ascorbic acid content to nearly the original values during the remainder of the storage period of 16 mo. Samples fortified with ascorbic acid decreased in ascorbic acid content for about 12 mo. and then increased up to higher than the original values at the end of 20 mo. of storage. Henry *et al.* (6) reported a 20 per cent loss in ascorbic acid (total) during spray drying and no change in ascorbic acid content of powders after 7-mo. storage in a cool dry place with gas-packed samples (75 per cent, 25 per cent CO_2). There was about 20 per cent more loss thereafter up to 1 yr. of storage. Findlay *et al.* (4) found the ascorbic acid in whole milk powders decreased rapidly at first (1 to 1.5 mo.) and more slowly thereafter. The loss was much more pronounced at 47 than at 15° C. storage.

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[1] Scientific paper no. 978, Washington Agricultural Experiment Stations, Institute of Agricultural Sciences, The State College of Washington, Pullman.

[2] American Dairy Association Research Grant in Cooperation with the Washington State Dairy Products Commission.

EXPERIMENTAL PROCEDURE

Unselected milk from the college herd was preheated at 170° F. for 30 min. and condensed in 20-gal. lots to approximately 40 per cent total solids in a 16-in. Rogers pan at 24 to 25 in. of vacuum. The concentrate was homogenized twice at 2,000 and 500 lb. pressure in a Manton-Gaulin two-stage homogenizer of 25-gal.-per-hour capacity. All the processing equipment in contact with the milk was stainless steel except the tinned containers in which it was received or handled during processing.

Each lot of concentrated milk was divided into four portions and given the following four treatments: (a) Control, no treatment; (b) additions of *l*-ascorbic acid at the rate of 100 mg. per liter of reconstituted milk (7 parts water to 1 part powder); (c) addition of *l*-ascorbic acid at rate of 100 mg. per liter plus 0.1 per cent sodium citrate calculated on the reconstituted basis; and (d) 0.1 per cent sodium citrate on reconstituted basis. The experiment was repeated four times during 1 mo. with the four variables, making a total of 16 powders.

The milk was condensed and dried the same day, and the drying was done in an experimental pilot plant drier similar in design to that used by Coulter (2). Within an hour after drying, the powders were packed in no. 2 flat tins containing about 120 g. per can. The samples were stored at 85° F., and approximately one-half the cans were double nitrogen-packed as follows: The tins were punctured with a nail and subjected to a pressure of 1 mm. or less in a vacuum chamber for 2 hr., nitrogen was allowed to run back at a slight positive pressure and the tins were soldered. The tins were allowed to desorb for 48 hr. and the process repeated.

The moisture content of the powders was determined by the vacuum-oven method. Since it is very difficult, if not impossible, to get the same moisture content for all powders in a series, a range of moisture levels was obtained for each series consisting of one powder slightly below 2.0 per cent moisture, two between 2.0 and 3.0 per cent and one slightly above 3.0 per cent.

Powder samples were stored at 85° F. and removed after 0, 1, 2, 4, 6, 8 and 12 mo. for determination of flavor, ascorbic acid, peroxides, ferricyanide-reducing groups, oxidation-reduction potential (Eh) and hydrogen ion concentration (pH).

The flavor scoring was done by a panel of six people, five of whom had 2 to 3 yr. previous experience judging whole milk powders. The powders were reconstituted on the basis of one part of powder to seven parts of distilled water and scores were assigned as follows: 9–10, no defect, equivalent of fresh whole pasteurized milk; 8–9, no defect except possibly very slight typical heated flavor; 7–8, slight heated or off-flavor not readily definable; 6–7, slightly oxidized or stale or both; 0–6, progressively more oxidized or stale, or foreign, rancid, etc. Scoring was in 0.5 points above 5 and in full points below 5. Averages were taken of the samples as judged by the panel.

The reduced ascorbic acid was determined by the method of Josephson and Doan (9). Peroxide formation evaluated by the method of Loftus-Hills and Thiel (7) and potassium ferricyanide-reducing groups were determined by the method of Chapman and McFarlane (1) as modified by Crowe *et al.* (3).

The oxidation-reduction potential and hydrogen ion concentration were both determined with a Beckman Model G and Model M potentiometer. A platinum electrode was used for the former determinations and a glass electrode for the latter.

EXPERIMENTAL RESULTS

Table 1 gives the mean values of all chemical and flavor tests made on the samples studied during a storage period of 1 yr. at 85° F. in air and nitrogen pack. Table 2 is derived from table 1 and gives the interpolated results showing the loss or gain in average analysis values at the storage time corresponding to a loss of 1.0 point in flavor score, which represents the onset of oxidized or stale flavor in the samples.

The findings for the whole milk powders in air and nitrogen packs at 85° F. are as follows:

Ascorbic acid. As shown in table 1, the ascorbic acid loss is greatest during the first month, decreases at a slightly slower rate up to 4 mo. of storage, and then much more slowly up to 12 mo. of storage. Nitrogen packing preserves the ascorbic acid content and the losses are quite small, but the general pattern of ascorbic acid destruction is the same. Where 0.1 per cent sodium citrate was added to the powders, there definitely is a greater ascorbic acid retention during storage. These results indicate that sodium citrate may have a slight synergist effect on ascorbic acid. Assuming ascorbic acid to be a synergist itself, as classified by Mattill (11), a situation of a synergist for synergist exists.

Oxidation-reduction potential (Eh). The Eh of the reconstituted powder samples stored at 85° F. increases in a linear fashion for the first 4 mo. levels out and reaches a peak at about 6 mo. and decreases slightly up to 12 mo.

The addition of 100 mg. of ascorbic acid per liter of reconstituted milk lowers the Eh values, and the addition of 0.1 per cent sodium citrate alone and with ascorbic acid also decreases the Eh slightly. Nitrogen packing reduces the rate of increase of Eh in storage and lowers the level which it attains before decreasing. Air-packed and nitrogen-packed samples exhibit off-flavor development at approximately the same Eh values (tables 1 and 2). In the air-packed samples, about 3 mo. are required to reach the critical Eh exhibited by a flavor loss of 1.0 in score. With nitrogen pack, 8 to 12 mo. are required to reach the same Eh value and resulting off-flavor formation.

The initial Eh values, as well as their increase which resulted in off-flavor development, varied with the treatment given. The initial Eh can be lowered by the addition of ascorbic acid or sodium citrate or both without the initial Eh value influencing the development of off-flavor materially.

Peroxide formation. The peroxide values are given in table 1 for the control samples and for samples with added sodium citrate. The latter samples had slightly lower peroxide values. The samples containing added ascorbic acid resulted in enough ascorbic acid dissolving in the benzene-methanol to give color interference in the test of Hills and Thiel (7). Neutralization of the ascorbic acid with indophenol dye was unsuccessful. Further work is needed to adopt this test to whole milk powders containing added ascorbic acid.

Hydrogen ion concentration (pH). The pH of the reconstituted whole milk

TABLE 1

Average analysis values[a] of whole milk powders with and without added ascorbic acid and Na citrate stored at 85° F.

Treatment	Moisture content	Storage age	Ascorbic acid		Eh values		pH		Ferricyanide[c] values		Peroxide[d] values		Flavor scores	
			Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2
		(*mo.*)	(*Mg./l.*)		(*volts*)									
Control	2.63	0	10.35	10.35	+0.336	+0.336	.....	.....	8.2	8.2	0.0207	0.0207	8.0	8.0
		1	7.9	8.4	0.342	0.339	6.63	6.62	8.6	8.7	0.0419	0.0327	7.6	7.9
		2	7.2	9.4	0.352	0.345	6.63	6.64	8.1	8.2	0.0777	0.0303	7.5	7.8
		4	6.9	8.9	0.363	0.358	6.54	6.53	8.0	8.4	0.1679	0.0917	6.2	7.7
		6	6.8	9.3	0.379	0.367	6.58	6.57	7.4	8.2	0.2411	0.0935	5.9	7.6
		8	6.5	8.8	0.363	0.358	6.54	6.57	11.0	11.4	0.3075	0.1042	5.2	7.1
		12	6.2	9.2	0.370	0.355	6.53	6.52	11.5	12.2	0.5420	0.0530	4.9	6.6
100 mg.[b] ascorbic	2.46	0	88.3	88.3	0.241	0.241	.....	.....	16.0	16.0	.....	.....	7.7	7.7
		1	68.1	85.2	0.263	0.256	6.59	6.60	14.6	15.5	[e]	.....	7.8	8.0
		2	66.2	85.0	0.274	0.267	6.60	6.59	13.7	15.7	.....	.....	7.8	7.7
		4	52.7	77.4	0.299	0.285	6.47	6.48	12.9	11.0	.....	.....	6.3	7.5
		6	49.9	84.9	0.309	0.289	6.54	6.55	11.7	14.6	.....	.....	6.3	7.4
		8	51.2	77.2	0.299	0.288	6.52	6.51	16.8	20.5	.....	.....	5.9	7.1
		12	49.2	80.8	0.292	0.273	6.48	6.48	18.3	22.5	.....	.....	5.6	6.9
100 mg. ascorbic 0.1% Na citrate	2.25	0	93.8	93.8	0.231	0.231	.....	.....	16.8	16.8	.....	.....	7.3	7.3
		1	79.8	95.5	0.252	0.250	6.78	6.77	15.7	17.1	.....	.....	7.5	7.8
		2	76.8	91.1	0.258	0.254	6.78	6.79	15.3	15.8	.....	.....	7.9	7.8
		4	64.6	91.7	0.281	0.269	6.66	6.67	10.7	12.5	.....	.....	7.2	7.5
		6	61.6	92.2	0.286	0.271	6.72	6.73	12.7	15.6	.....	.....	6.4	7.3
		8	56.4	80.4	0.290	0.280	6.69	6.69	17.7	20.5	.....	.....	6.1	6.8
		12	55.6	86.7	0.282	0.276	6.66	6.68	17.6	22.4	.....	.....	6.1	7.1
0.1% Na citrate	2.26	0	13.5	13.5	0.323	0.323	.....	.....	11.1	11.1	0.0127	0.0127	7.5	7.5
		1	10.9	13.9	0.312	0.309	6.79	6.78	10.8	11.4	0.0311	0.0239	7.5	7.6
		2	12.6	14.3	0.330	0.314	6.82	6.84	10.4	11.2	0.0867	0.0250	7.7	7.8
		4	9.3	11.3	0.340	0.337	6.69	6.70	10.1	10.6	0.1242	0.0271	6.4	7.3
		6	9.4	12.1	0.348	0.333	6.77	6.75	8.9	9.7	0.1828	0.0508	6.4	7.2
		8	9.2	13.0	0.351	0.342	6.75	6.73	13.3	14.1	0.2993	0.0643	5.2	6.7
		12	8.8	12.1	0.346	0.344	6.69	6.69	14.7	14.9	0.4110	0.0095	5.6	6.7

[a] Each value represents an average of 4 replicate powders for each treatment and storage age.
[b] Per liter of reconstituted milk on a basis of 7 parts of water to 1 part of powder.
[c] Moles of potassium ferricynide × 10-6 reduced / g. of powder.
[d] Milliequivalents of oxygen/kg. of powder.
[e] The addition of ascorbic acid to whole milk powders causes color interference in the peroxide test of Hills and Thiel (7).

TABLE 2

Interpolated analysis values showing loss or gain from original values at point of a loss of one in flavor score of powders

Treatment	Age at 1.0 point loss in flavor score		Ascorbic acid		Eh		pH		Ferricyanide values		Peroxide values	
	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2
	(*mo.*)		(*mg./l. reconstituted product*)		(*volts*)				($M \times 10^{-6}$ *g. of powder*)		(*M.e. O_2/kg. powder*)	
Control	3	8	−3.3	−1.3	+0.022	+0.022	−0.04	−0.06	−0.6	+2.9	0.1227	0.1042
100 mg. ascorbic added	3	12	−18.3	−7.0	+0.046	+0.047	−0.04	−0.09	−2.7	+4.5	.....	.....
100 mg. ascorbic plus 0.01% Na citrate added	5	12	−30.8	−13.4	+0.053	+0.049	−0.19	−0.19	−2.7	+5.6	.....	.....
0.01% Na citrate added	3	7	−2.5	−0.50	+0.012	+0.015	−0.05	−0.04	−0.8	+0.9	0.0927	0.0448

powders studied was lowered slightly by the addition of 100 mg. of ascorbic acid and raised by the addition of 0.1 per cent sodium citrate and combined ascorbic acid and sodium citrate. It was unaffected by the type of packing, whether air or gas.

During the storage, the pH of all powder samples decreased, regardless of the treatment or type of pack.

Ferricyanide-reducing values. Potassium ferricyanide-reducing values decreased during storage at 85° F. up to about 4 mo. in nitrogen packs and up to 6 mo. in air packs. Thereafter, up to 12 mo. they increased quite rapidly, exceeding the original values at 6 to 8 mo. storage. The rise in ferricyanide values coincides with a leveling out of Eh values and flavor scores.

Adding 100 mg. of ascorbic acid approximately doubles the potassium ferricyanide-reducing values. The addition of 0.1 per cent sodium citrate increases the ferricyanide reducing groups slightly, due possibly to its synergist effect in preserving ascorbic acid. Statistical analysis (not shown) showed that the range of moisture levels of the five replicate powders from slightly below 2 to slightly above 3 per cent did not materially influence ferricyanide-reducing values.

Flavor. The control samples, the sample to which 100 mg. of ascorbic acid per liter of reconstituted milk were added and the sample to which 0.1 per cent sodium citrate was added on a reconstituted basis all exhibited a flavor score loss of 1.0 point in 3 mo. in air pack and about 8 mo. in nitrogen pack. The sample containing 0.1 per cent added sodium citrate plus 100 mg. of ascorbic acid exhibited this flavor score loss in 5 mo. in air packs and 12 mo. in nitrogen packs. The addition of 0.1 per cent sodium citrate gave a slight salty flavor in all cases, which resulted in some of the flavor score loss.

However, the main flavor criticism for samples containing added ascorbic acid or sodium citrate was a stale, rather than the more typical oxidized flavor which was apparent in the control sample that was air packed. The two flavors are at times difficult to distinguish and may in some cases be a blend.

Nitrogen packing. Nitrogen packing greatly preserves the flavor and the ascorbic acid content, increases the ferricyanide-reducing values, reduces the peroxide values to practically nil, reduces the rate and limit of Eh development and has little effect on the pH.

DISCUSSION

The importance of Eh in the development of an intermediate oxidation flavoring compound in milk has been stressed by Greenbank (5). The development of this intermediate flavoring compound may be inhibited by the addition of either oxidizing or reducing agents to the milk before the flavor has developed.

Krukovsky and Guthrie (10) demonstrated the relationship of oxidized (tallowy) flavor to the pressure of ascorbic acid to dehydroascorbic acid. They state there is a critical range for the development of oxidized flavor. Greenbank (5), using a portion of the same data, correlated flavor scores with the ratio of dehydroascorbic acid to total ascorbic acid. He found that when less than 40 per cent of the ascorbic acid is oxidized, the flavor score is perfect; when up to 67 per cent is oxidized, the flavor becomes progressively worse; and with over 70

per cent oxidation the flavor improves and becomes as good as the original flavor. Table 1 shows that all nitrogen-packed samples have less than 40 per cent oxidation of ascorbic acid and that all the air-packed samples are in the critical range of 40 to 67 per cent oxidation.

Total destruction of the ascorbic acid by Krukovsky and Guthrie (10) and deaeration of the milk by Sharp *et al.* (13) also prevent oxidized flavor in milk. These are essentially methods of changing the oxidation reduction potentials of milk as well.

Saal and Heukelom (12) found that the oxidized flavor can occur in milk over a range of initial oxidation reduction values, but the flavor once developed is proportionate to the increase in oxidation reduction potential. Whenever Eh is decreased by treatment after a rise in value, the oxidized flavor tends to disappear. They believe the development of oxidized flavor in milk may occur in two phases. "In the first phase chiefly ascorbic acid is oxidized and only a little oxidizing substance is formed." "In the second phase the oxidizing substance and with it the flavoring substance is chiefly formed, while the remnants of ascorbic acid are further oxidized."

The results of present study on whole milk powder would tend to agree with the work done by the latter authors on fluid milk; however, the powder being in the dry state requires a number of months in storage to go through the same pattern of development. First, the rate of destruction of ascorbic acid is greatest in the first month or so of storage, with a gradual leveling off during storage. Second, with the several treatments used in the present study a range of initial Eh values was obtained, and the rise in Eh values from the initial values which was necessary to produce off-flavors varied with each treatment. However, samples with the same treatment when air-packed and nitrogen-packed demonstrated the same flavor score loss at about the same Eh values, although the nitrogen-packed samples required about 12 mo. in storage at 85° F. to reach the same Eh values as air-packed samples did in 3 to 5 mo.

Where Eh values leveled off, the flavor tended to deteriorate less rapidly. This may account for some cases in which flavor scores have leveled off or even improved in certain instances during later stages of storage. Although no oxygen contents were run, this leveling off period would probably coincide with exhaustion of the oxygen in the can. The character of the off-flavor varied with the treatment and, consequently, so did the initial Eh value. With additions of ascorbic acid or sodium citrate, or both, the off-flavor tends to be more stale than tallowy. The role played by ascorbic acid seems to be its influence on the oxidation reduction potentials of the milk powder, which in turn influences the character and degree of off-flavor. Although the changes are small, the increase in oxidation reduction potential during storage of whole milk powders may offer a possible method of determining the storage life as well as following the off-flavor development, provided the processing and storage history of the sample is known.

SUMMARY

The addition of ascorbic acid or sodium citrate at levels of 100 mg. and 0.1 per cent, respectively, per liter of reconstituted milk, did not materially prolong

the storage life in air pack of whole milk powders at 85° F. storage. Sodium citrate has a slight synergist effect on ascorbic acid by preserving it in powder samples stored at 85° F. and, when combined with ascorbic acid, increased the storage life of the powder from about 3 mo. to a period of 5 mo. over the control or ascorbic acid alone. The predominating flavor defect was staleness rather than tallowiness as in the control samples, and slightly salty where 0.1 per cent sodium citrate was used.

The degree of off-flavor development was dependent upon the developed Eh values. Powder samples with the same treatment, air-packed and nitrogen-packed, developed off-flavor score losses of 1.0 point at about the same Eh values. The latter, however, required about 9 mo. longer in storage to reach these critical Eh values, which varied with the treatment given.

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THE MANUFACTURE OF MOZZARRELLE CHEESE FROM PASTEURIZED MILK

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A cheese type, partially described by Marquardt (4), which rapidly is gaining importance in this country is a soft, slightly ripened cheese from whole or partly skimmed milk, known as Mozzarrelle. This cheese which for years has been among the staple diet of the Italian people is now being consumed in large quantities by many persons in the United States irrespective of their nationalistic descent. In New York State and surrounding areas an important industry, utilizing annually millions of pounds of whole milk, has developed from the manufacture of this cheese.

A more complete description of this cheese would indicate that until recently it was manufactured from raw milk. The raw milk for this cheese, is set at 88 to 90° F. with rennet but no starter and after the curd is cut or broken, these curds are not cooked but are allowed to mat slightly. Then the whey is quickly drained from the vat and the matted curds washed with cold water and placed in cheese cloth or muslin sacks. These bags of curd are placed on wooden floor racks of a cold room and well iced. The following days the bagged curd is moved to higher temperature rooms during daylight hours to insure proper ripening. After 2 to 6 days, depending on the curing room temperature, the curd reaches the optimum peak of ripening and can be consumed as such or may be processed. This ripening and processing which follows is usually not carried out in the manufacturing plant but at some small sub-dealer located in the city where the cheese is to be consumed. Only small quantities usually are ripened at any time, the extent depending upon the prospects for immediate sale.

Processing of the ripened cheese is accomplished by cutting up the matted, ripened curd into small cubes and placing them in hot water (160 to 180° F.) for a few minutes, after which the curd, now stringy if properly made, is pulled and molded by hand. Processing conditions and procedures at the sub-dealers are extremely unstandardized. The finished product, if made from raw milk, has a satiny smooth, well knit body and invariably has a rancid flavor. This latter flavor was not considered desirable by the producers of this cheese. The hand-molded cheeses if shaped like a loaf of Vienna bread or a brick are called Mozzarrelle, whereas if shaped like a tulip bulb with protruding ears are called Scarmoza, but the classification is loose. Brine salting of the molded processed Scarmoza cheese invariably completes the procedure. The storage life of the finished cheese, even at low temperatures, probably does not exceed 3 wk.

As a basis for "Pizza pie" the Mozzarrelle cheese is placed in a bread slicer and slices of cheese approximately $\frac{1}{8}$ to $\frac{1}{4}$ in. are obtained. These slices are placed on the surface of a special type unbaked bread dough residing in a large pie pan.

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Then a tomato sauce, with or without meat and flavoring compounds, is spread over the cheese. The open pie next is placed in an oven for approximately 10 min. at 500° C. It is essential in this preparation that the crust does not burn before the cheese is fully melted and it also is essential that the cheese become stringy but not too tough after being cooked. However, many other uses, other than Pizza pie are available for this type of cheese.

It was due primarily to the fact that some of this cheese is consumed before and after the processing in hot water without being made into Pizza and also because of extremely variable processing temperatures employed some of the cheese have shown phosphatase positive reactions, that the Public Health Department of a large municipality in New York State began enforcing a local ordinance in June, 1950, requiring that all Italian cheese curd shipped into their city must be made from properly pasteurized milk. Non-compliance with this ordinance resulted in embargoes whereby shipments of raw cheese were confiscated.

This enforcement problem existed up to about late August, 1950, as practically all the Italian cheese manufacturers supplying this area insisted from practical experience, that it was technically impractical, if not impossible, to manufacture suitable Mozzarrelle cheese from properly pasteurized milk. Industry spokesmen maintained that conversion over to pasteurized milk produced a number of processing problems which were not encountered in the conversion of other types of cheese, such as cottage or cheddar. Faults attributed by these manufacturers to pasteurized-milk Italian cheese curd made without starter included the following: (a) Curd usually was proteolyzed and rotted; (b) stretching of the curd, which is necessary during processing for proper molding, was eliminated; (c) heavy losses in yield occurred; and (d) Pizza pies made with cheese were of poor quality.

Suggestions have been made that the low bacteria count in properly pasteurized milk is not sufficient to bring about correct ripening of the curd and that the addition of commercial lactic acid starter would solve this problem. Italian cheese makers stated however that the use of commercial lactic starters is undesirable in that overripening occurs very quickly causing sour odors and poor physical stretch of the curd and the cheese curd yield is low.

At the recent request of the public health officials of the large municipality (3) and a number of Italian cheese manufacturers, the Department of Dairy Industry, Cornell University, was asked to investigate this problem. This report (2) in a preliminary form dealing with DK starter was presented earlier to this group of health officials and cheese makers. The objective of this study did not include the physiochemical fundamentals of this cheese, even though there is great need for information in this direction, but was limited to modifications which might quickly resolve the immediate problem facing the industry, *i.e.,* how to make Mozzarrelle cheese from pasteurized milk which would have consumer acceptance. Two approaches were studied:

(a) The effect of varying concentrations of commercial lactic starter upon the quality of pasteurized milk Mozzarrelle cheese and (b) the effect upon the quality of pasteurized milk Mozzarrelle cheese of DK (*S. faecalis*) starter as first used by Dahlberg and Kosikowsky (1) for cheddar cheese.

EXPERIMENTAL METHODS

This study was conducted both in the laboratory and at various commercial Italian cheese plants throughout New York State. In the laboratory into each of five small cheese vats were placed 260 lb. of whole milk pasteurized to 161.5° F. for 16 sec. In a sixth vat, 260 lb. of raw milk were added. The raw milk was set with 15 ml. of rennet and no starter, whereas the pasteurized milk was set with 15 ml. of rennet and various amounts of active commercial lactic starter or DK starter depending on the nature of the experiment. The curd was cut with 0.75-in. knives and after whey drainage the curds were washed with 55° F. water for 5 min. The cheese curd was bagged, then placed on floor racks in a cold room at 40° F. and cooled until the next day. In the days following the bagged curd was brought out and warmed to temperatures close to 80° F. for 4- to 8-hr.

TABLE 1

The manufacture of Mozzarelle cheese from pasteurized milk with commercial lactic acid starter

Vat no.	Cheese milk[a]	Per cent lactic starter	Quality of cheese curd after 24 hr. at 40° F.		pH of curd after 24 hr. in cold room (40° F.)
			Flavor	Body	
1	Raw	none	good	green	6.00
2	Pasteurized	0.2	sour	overripe	5.05
3	Pasteurized (1 hr wait at 88° F. between starter addition and renneting.)	0.2	sour	overripe	4.90
4	Pasteurized	1.0	sour	overripe	4.95
5	Pasteurized (1 hr wait at 88° F. between starter addition and renneting.)	1.0	sour	overripe	4.90
6	Pasteurized	3.0	sour	overripe	4.80

[a] Milks pasteurized to 161.5° F. for 16 sec. All milk from same blend and was of average commercial quality.

periods in order to simulate ripening conditions as practiced by many sub-dealers of this cheese. In the evening the curds again were cooled below 40° F.

pH measurements were made directly on the cheese curd by means of a Beckman (laboratory model) glass electrode potentiometer.

The physical quality of the cheese and its flavor were judged independently by qualified representatives of the Italian cheese industry, who processed much of the curd, and by the author. The term overripe listed in the paper at various stages is one commonly used in the Italian cheese industry as referring to a cheese having an acid taste and one whose body has passed the critical physical stretch phase and is no longer able to be molded.

EXPERIMENTAL RESULTS

Mozzarelle cheese from pasteurized milk with commercial lactic starter. Pasteurized milk in this experiment contained active commercial lactic acid starter ranging from 0.2 to 3.0 per cent concentration. Table 1 shows the re-

sulting quality of this cheese. In all cases where the pasteurized milk cheese was made with commercial lactic starter, even in the low concentration of 0.2 per cent, the resulting curd was sour and unsuitable for sale after 24-hr. storage at 40° F. This overripened condition invariably has been considered by Italian cheese makers as characteristic of Mozzarelle cheese when commercial lactic starter was used, irrespective of the concentration added. It also is interesting to note that the pH of cheese made from milk with commercial lactic acid starter was very low, ranging from 4.8 to 5.1. On the other hand, the control cheese made from raw milk without starter had a pH of 6.0 after 24 hr. at 40° F. No significant differences appeared to exist here between cheeses made from milks in which the starter was held at 88° F. for 1 hr. before renneting and those milks in which renneting coincided with the addition of the starter.

Mozzarelle cheese from pasteurized milk with DK starter. In another series of experiments in the laboratory, pasteurized whole milk was made into Mozzar-

TABLE 2

The manufacture of Mozzarelle cheese from pasteurized milk with DK starter

Vat no.	Cheese milk[a]	Per cent DK starter	Quality of cheese curd after 24 hr. at 40° F.		pH of curd after 24 hr. in cold rm. (40° F.)	Quality of cheese curd in 5 d. at intermittent temp. 40–80° F.	
			Flavor	Body		Flavor	Body
1	Raw	none	excel.[b]	green	6.10	excel.	good
2	Pasteurized (1 hr.wait at 88° F. between starter addition and renneting.)	0.5	excel.	green	6.01	excel.	excel.
3	Pasteurized	1.0	excel.	green	6.00	excel.	excel.
4	Pasteurized	2.0	excel.	green	6.07	excel.	excel.
5	Pasteurized	3.0	excel.	green	5.90	excel.	good
6	Pasteurized	none	excel.	green	6.55	poor	green

[a] Milk pasteurized to 161.5° F. for 16 sec. All milk from same blend and was of average commercial quality.

[b] Excel. = Excellent.

relle cheese employing the same procedure as before but using quantities of active DK starter, ranging from 0.5 to 3.0 per cent, instead of commercial lactic starter. The resulting cheese, after being cooled overnight at 40° F., was brought out to room temperature (approximately 80° F.) daily for about 4 to 8 hr. until they were fully ripened which was about 5 to 6 days.

Table 2 shows the effect of DK starter upon the body and ripening quality of the cheese and also upon the pH. From the comments listed by an expert Italian cheese maker it may be noted that all the cheese with DK starter ripened well and were comparable to the raw milk control. Also, it may be seen that the pH of the samples after 24 hr. compared very similarly with that of the raw milk curd. (pH 6.0 to 6.1.)

Commercial trials with DK starter. Several large batches of Mozzarelle cheese made from milk pasteurized to 161.5° F. for 16 sec. using 0.2 to 1 per cent DK starter were made at an Italian cheese factory and shipped to city markets.

Checks on the reception of this cheese showed that the processors did not notice any physical differences between their raw milk cheese without starter and the pasteurized milk cheese containing DK starter. Yields from the latter were found to be as good as those from raw milk.

To further check the quality of the pasteurized milk cheese with DK starter relative to Pizza pie making operations, a number of cheeses of this type were made and ripened at the cheese factory and brought to an Italian restaurant to be made into Pizza pies. Table 3 lists the comments of the Italian chef who was unaware of the history of the cheeses. It is evident from his observations that pasteurized-milk Mozzarelle cheese with DK starter will make good Pizza pie.

TABLE 3

The quality of "Pizza pies" from Mozzarelle cheese made of properly pasteurized milk with DK starter

Vat no.	Cheese milk[a]	Per cent DK starter	Age of cheese curd before processing[a]	Quality of pie		
				Flavor	Body	Meltdown
			(*d.*)			
1	Raw	none	3	good	hard	good
2	Pasteurized plus 0.01% $CaCl_2$	0.7	2	good	sl. hard	good
3	Pasteurized	1.0	3	excel.	good	excel.
4	Pasteurized 0.02% $CaCl_2$	0.7	2	good	sl. hard	good
5	Pasteurized	0.7	4	good	good	good

[a] Cheese made by Valley View Creamery, Rochester, N. Y. from their own whole milk of high quality and pasteurized at 161.5° F. for 16 sec. When near peak of ripening cheese curd was heat processed in 180° F. water and used for Pizza pies at Lehigh Valley Restaurant, Ithaca, N. Y. Cheese pies were full size and contained tomato. No. 5 contained tomato and sausage. All comments on quality by Italian chef and were based on characteristics of cheese just after being taken from hot oven (500–550° F.). Pie is held in hot condition by placing in warming box until purchased by consumer.

DISCUSSION

As this study on Mozzarelle cheese progressed it soon became apparent that many of the principles underlying its proper manufacture were not clearly defined. To some extent this condition was due to the natural reticence in the past of these manufacturers to elaborate on their procedures in publication, but in the main it was due to the limited fundamental research available concerning the properties of this cheese. There is a great need for further information regarding the basic characteristics of this type of Italian cheese. A start in this direction has been undertaken by Marquardt (4). The latter investigator has indicated in one phase of his work that under controlled conditions at one cheese plant it was possible to successfully make Mozzarelle cheese from pasteurized milk without starter. All the curd was processed or worked at this plant.

Apparently then, Mozzarelle cheese of satisfactory quality can be made under controlled conditions from pasteurized milk without starter but its continued quality would be contingent on the fact that enough of the proper type of natural

bacteria, most likely *S. faecalis,* survive to later reduce the pH to the proper point for optimum ripening and that very few of the proteolytic type of bacteria be present in the pasteurized milk.

In actual practice, however, most of the Italian cheese makers in this area have found it very difficult to make satisfactory Mozzarrelle cheese from pasteurized milk without starter as the required type of natural bacteria in optimum concentration have seldom materialized in the pasteurized milk and, as a result, the curd failed to ripen properly and also produced disagreeable proteolytic flavors and a slimy body according to its manufacturers. As there is a wide range of quality in milks used for this type of cheese, for example a good deal of this product is made from high quality diverted milk, assurance was needed by these cheese makers that enough proper bacteria would be present from day to day in the pasteurized milk. Adding commercial lactic starter did not alleviate this condition but actually created additional problems of a more acute nature. The reports of experiences of the Italian cheese makers in this connection were confirmed by this study.

Mozzarrelle cheese curd is characteristically very wet and low in acid. These characteristics may serve to explain why regular commercial lactic starter, regardless of the concentration, cannot be used satisfactorily. This type of starter produces acid at a very rapid rate between 90 and 70° F. As it may take the bagged curd a number of hours to reach a temperature of 50° F. where acid production for this starter would be slow it is apparent, that in this period, fermentation of the lactose proceeds at a rapid rate. Based upon certain fundamentals involved in cheese making it might be reasonable to make an assumption that in this case as the lactic acid increases in concentration in the whey, more and more buffering salts are put into solution and as there is a constant drainage of this whey in storage many of these salts are leached from the curd. Consequently, due to the loss of these critical buffering salts the pH continues to drop rapidly and overripening results in a very short time.

On the other hand, because of the different lactic acid production properties of DK starter, this condition would not exist. DK starter produces sufficient rates of lactic acid at 90° F. but the rate of acid production is slowed markedly below 80° F. Consequently, the whey does not become too acid while the curds are cooling and, as a result, more buffers are evidently retained in the curd. Upon later ripening in a day or two at higher temperature when the curd is drier, more buffer salts, such as the phosphates, are available to prevent the pH from dropping sharply when fermentation of the lactose is resumed by the *S. faecalis* bacteria and to more easily maintain the pH at a proper level for controlled ripening.

In processing cheese of this nature and in accordance with the early findings of VanSlyke and Bosworth (2), the proper stretch of the curd apparently is attained only when sufficient monocalcium paracaseinate is made available in this curd by lactic acid. The concentration of this compound is directly related to the pH of the curd up to a point. At a certain pH which as indicated by Mar-

quardt (3) most likely falls between 5.5 to 5.3 but which may be higher for some samples, optimum stretch is realized. This stretch which can be compared to the strings obtained on a hot iron test for cheddar cheese does not develop to any great extent above or below these critical pH values. Because of this it is important that underripening or overripening does not occur at time of processing.

Many of the large Italian cheese companies in New York and Pennsylvania have been using this new starter since the introduction of the preliminary report (2). They have found, based on experience, that a concentration of 0.20 to 0.50 per cent DK starter or about 20 to 50 lb. to 10,000 lb. milk produces the best cheese.

If upon further application the use of DK starter continues to prove successful in commercial operations, more and better control of the cheese-making operation may be attained, since ripening can proceed on a regular time schedule. The flavor of the product should be, if a good starter control program is instituted, of finer quality as there should be little rancid, unclean flavor development from the pasteurized product. With greater uniformity in the quality of this product, more cheese of this nature should be made. In addition, the increased use of pasteurized milk for this type of cheese will minimize public health problems which in the past have made this cheese subject to criticism by state and city health officials.

SUMMARY

In this study, Mozzarrelle cheese made from properly pasteurized milk without starter did not ripen well. When commercial lactic acid starter was used in pasteurized milk, even in minute quantities, extensive souring and overripening of the cheese took place. Regular commercial lactic acid starter apparently is not feasible for the manufacture of this type of cheese.

Cheese of the Mozzarrelle type made from properly pasteurized milk with DK starter ripened as well as the raw milk cheese controls. The flavor and yield from these pasteurized milk cheeses were good and Pizza pies made with them were of excellent quality. Concentrations of from 0.20 to 0.5 per cent DK starter appear to be adequate for commercial operations.

More fundamental information concerning the physio-chemical nature of this cheese would be extremely useful. In addition, ways and means to achieve standardization of procedure, particularly with the sub-dealer and the establishment of standards of identity should prove valuable to the industry.

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SEASONAL VARIATION IN THE VITAMIN A POTENCY OF OREGON-PRODUCED BLUE VEIN CHEESE[1, 2, 3]

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The vitamin A potency of blue vein and roquefort cheese has been variously reported to range from less than 3,400 I. U. to more than 18,000 I. U. per pound (1, 2, 3, 4). Watt and Merrill (2) in their recent table on the composition of foods indicated 5,630 I. U. per pound for blue vein cheese. They denote that this is an imputed value. The fact that differences have been reported in the literature is not surprising in that the vitamin A potency of milk fat will vary due to seasonal as well as regional feeding practices. The average vitamin A potency of winter milk has been reported to be about 60 per cent that of summer milk (5). This difference may be greater in certain instances but it is hard to comprehend that the range from less than 3,400 to over 18,000 I. U. per pound is due to seasonal sampling or regional feeding practices. Consequently, a study has been made of the vitamin A potency of ripened, blue vein cheese as produced in Oregon.

EXPERIMENTAL AND RESULTS

Samples of blue vein cheese were obtained each month. This cheese had been previously ripened and stored at the cheese makers for a period of 5 mo. under normal factory conditions. The age of the cheese was that which normally was sold to the consumer. Upon receipt at the laboratory, the samples immediately were placed in the refrigerator prior to analysis. The methods of analysis used for fat and moisture were those of Wilster *et al.* (6). Vitamin A and carotene were determined in accordance with the procedure as suggested by the Technical Committee in Charge of the Nation Wide Survey (5, 8).

The results of this study are summarized in table 1. The vitamin A potency of blue vein cheese varies from about 5,600 I. U. to 9,500 I. U. per pound, depending on the month of manufacture. The average vitamin A potency in this case was found to be 7,225 I. U. per pound. As was expected, the lower values could be associated with the seasons of poorest pastures. The highest values were found in April and May when pastures are considered to be lush. The greatest apparent difference in the table may be noticed in the carotene values, which range from 8 to 9 γ per gram to approximately twice this amount. While the range in the concentration of vitamin A is not as great, it is of the same order so far as its physiological significance is concerned.

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2 The samples of blue vein cheese were obtained from the Langlois Cheese Makers, Langlois, Oregon.

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The samples as analyzed had been stored for a period of 5 mo. No effort was made, in this case, to determine whether the vitamin A potency was altered during storage. A study to determine the stability of this vitamin in cheddar cheese produced each month of the year and stored for periods up to 1 yr. is now in progress. It previously has been reported (9) that in cheddar cheese there is no serious destruction of this vitamin during ripening. Our results to date are in agreement with this conclusion.

That the vitamin A potency appears to be stable in ripening and storage of blue vein cheese may be deduced from the results of a previous butter survey made in the region of this cheese factory. It was found that the butter produced in this area ranged from about 13,000 I. U. to over 20,000 I. U. per pound (7). If the cheese fat is calculated on the basis of butter, the range would be

TABLE 1

Vitamin A potency of blue vein cheese produced in Oregon (July, 1949–June, 1950)

Mo. of manufacture	Fat	Carotene	Vitamin A	Potency[a]
	(%)	(γ/g. fat)	(γ/g. fat)	(I. U./lb.)
Jan.	31.75	9.35	5.50	5,650
Feb.	32.75	10.70	7.47	7,440
Mar.	33.50	9.93	7.17	7,200
Apr.	30.75	18.20	8.67	9,470
May	29.50	16.15	8.35	8,420
June	30.75	15.80	6.78	7,770
July	32.40	10.12	6.26	6,440
Aug.	30.50	8.16	6.32	5,630
Sept.	32.60	10.58	6.53	6,770
Oct.	31.00	12.26	7.32	7,320
Nov.	31.50	13.17	6.83	7,340
Dec.	32.00	11.58	7.17	7,270
Av.	31.58	12.16	7.03	7,225

[a] Corrected for loss of vitamin A in analysis.

from around 14,000 I. U. to about 24,000 I. U. per pound. Since these values are in excess of that which could be expected from the butter survey, it would seem apparent that little if any of the vitamin A potency of blue vein cheese is destroyed during ripening and storage for a period of 5 mo.

SUMMARY

The vitamin A potency of blue vein cheese as produced in Oregon ranged from about 5,600 I. U. to 9,470 I. U. per pound, depending upon the month of production. The average vitamin A potency per pound of blue vein cheese was found to be 7,225 I. U. It would appear that little if any vitamin A potency is lost during the ripening and storage of blue vein cheese.

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ANTIBIOTIC FEED SUPPLEMENT (AUREOMYCIN) FOR DAIRY CALVES

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It is now fairly well established that antibiotics *per se* and feed supplements containing antibiotics stimulate the growth of chickens (14, 15) and pigs (6, 9).

In regard to ruminants, several investigations (3, 4, 5, 8, 11) on feeding antibiotics recently have been reported. Evidence by Rusoff (11) indicated that small amounts of aureomycin supplement (2.27 g. aureomycin per 100 lb. feed) in a grain ration stimulated the growth of 3.5-mo.-old dairy calves during an 8-wk. period. Bartley *et al.* (3) have reported that aureomycin increased the growth of young calves up to 7 wk. of age by preventing scours. The calves were fed a normal diet supplemented daily with an aureomycin supplement (approximately 15 mg. aureomycin per 100 lb. body weight) by capsule. Loosli and Wallace (8) also have demonstrated that aureomycin produced a slight increase in growth rate in young calves up to 8 wk. of age and a decrease in the incidence and severity of diarrhea. The calves, 10 to 14 days of age, were fed various milk substitutes to which was added a 2.8 per cent level of aureomycin supplement, or 0.5 g. of crystalline aureomycin per 100 lb. of dry milk substitute. The animals also received grain and hay.

Detrimental effects of feeding aureomycin have been reported by Colby *et al.* (5) who have evidence that the antibiotics, aureomycin, penicillin and streptomycin (100 mg. levels daily), or aureomycin supplement (0.5 per cent level) caused lambs to go off feed, lose weight and have diarrhea. Bell *et al.* (4) also have reported adverse effects of feeding aureomycin (0.6 g. daily) to steers on a balance trial involving urea. The animals showed marked anorexia and diarrhea within 48 to 72 hr. Mild symptoms were observed when 0.2 g. aureomycin was fed daily. On the basis of the last two reports, it was suggested that rumen function was inhibited and, therefore, the use of antibiotics or antibiotic feed supplements in ruminant feeding has not been advocated. The present study reports the beneficial effects of an aureomycin supplement on ruminating calves over a 20-wk. period.

EXPERIMENTAL

Two equal groups of Jersey calves, each containing five males 14 wk. of age, were used. Both groups of calves had been weaned from milk at 28 days of age and had received a basal all-plant protein calf starter. In addition, one of the groups had received a vitamin B_{12} feed supplement, supplying 0.5 mg. vitamin B_{12} per 100 lb. feed, up to 14 wk. of age. The supplementation of vitamin B_{12} was without effect on the growth of calves (13). The basal group was continued on a simple grain ration consisting of one part cottonseed meal, three parts yellow

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corn meal, 2 per cent bonemeal and 1 per cent common salt. The other group was fed this basal ration plus 2 per cent of an aureomycin supplement (Aurofac[1]). The calves in both groups were fed 4 lb. of grain daily for the first 4 wk., 6 lb. daily for the next 4 wk. and 8 lb. daily thereafter for the remainder of the trial. On the basis of the amount of grain fed, the calves in the aureomycin group were receiving from 90 to 181 mg. of aureomycin daily. All animals were allowed limited pasture and some medium-quality lespedeza hay. The experiment was continued for 20 wk.

RESULTS AND DISCUSSION

The average daily gain per calf per day for each group at intervals of 2 wk. is shown in table 1. The calves in the aureomycin group showed an increase in

TABLE 1

Average daily gain (lb.) per calf

Group	No. of calves (Jerseys)	Weeks of age										
		14	16	18	20	22	24	26	28	30	32	34
Basal	5	0.79	0.92	1.04	1.10	1.42	1.40	1.54	1.70	1.68	1.69	1.66
Aureomycin (Basal + 2% Aurofac[b])	5	0.78[a]	1.48	1.42	1.44	1.53	1.50	1.59	1.73	1.61	1.65	1.65

[a] Results with vitamin B_{12} supplement.

[b] Supplied through the courtesy of E. L. R. Stokstad, Lederle Lab., American Cyanamid Co., Pearl River, N. Y.

weight over those in the basal group during the first 6 wk. on experiment (20 wk. of age) by approximately 60, 36 and 30 per cent, respectively, for the 2-wk. periods. The average daily gain at this time was 1.44 lb. for the aureomycin-fed calves and 1.10 lb. for the control calves. This difference was found to be significant at $P = 0.05$. After 8 wk. this increase in growth rate was approximately 8 per cent and declined gradually thereafter. After 20 wk. (34 wk. of age), the average daily gain per calf per day for both groups was similar, being 1.65 and 1.66 lb., respectively. Apparently, the stimulating effect of aureomycin on the growth of ruminating calves 14 wk. of age is only of short duration.

After 4 wk. on experiment, the calves receiving aureomycin had a smooth hair coat and sleek solid muscular appearance, while the calves in the basal group were rougher in appearance and had a larger middle. The better appearance of the antibiotic-fed calves persisted throughout the experimental period. At no time was there any evidence of anorexia or diarrhea.

While only a few animals were used in this investigation, the consistent beneficial response of the calves to the antibiotic, especially during the first 6 wk. of the 20-wk. period, is of significance. It is conceivable that the beneficial effect of aureomycin on the experimental animals was due to a synergistic action with extra-stored vitamin B_{12}, for these animals previously had received vitamin B_{12} supplementation during their first 14 wk. of life. Since the present experiment

[1] Formerly known as Lederle's A.P.F. no. 5; contained 2.5 mg. of aureomycin per g.

was completed, Rusoff and Davis (12) have obtained data which show that supplemental vitamin B_{12} is not necessary with aureomycin in order to obtain a growth stimulation in young calves. These workers fed calves crystalline aureomycin from 2 to 16 wk. of age, using a basal all plant-protein calf starter. Evidence is available that ruminants synthesize vitamin B_{12} by rumen or intestinal symbiosis (1, 7, 13) and, therefore, it is suggested that perhaps additional supplementation of vitamin B_{12} along with an antibiotic for calves might give a still greater growth response than the antibiotic *per se*.

The detrimental effect of antibiotics on lambs (5) may be due to a species difference, while the effect of aureomycin on steers (4) may be due to the maturity of the ruminants, the daily high level (600 mg.) of antibiotic fed, or to the type of ration used, since these workers were testing the digestibility of a ration containing urea. Perhaps the percentage of protein in the ration is involved.

Bell *et al.* (4) also reported that aureomycin decreased the digestibility of crude fiber by 50 per cent and suggested that the cellulytic microorganisms in the gastro-intestinal tract were affected. A bacteriological study of the rumen contents of young calves receiving aureomycin and no aureomycin was made by Alford and Rusoff (2). This investigation has failed to reveal any effect of aureomycin on the morphological types of microorganisms normally present in the rumen (10). These data suggest that some of the effects of aureomycin on dairy calves might be due to its action on the intestinal microflora. Considerable additional evidence should be obtained to verify such a theory.

SUMMARY

An aureomycin supplement (Aurofac) fed at a 2 per cent level in a grain ration stimulated the growth of ruminating dairy calves (14 wk. old) by approximately 35 per cent for the first 6 wk., and resulted in better appearance and condition of the animals. This increased rate of growth declined thereafter and after 20 wk. of feeding the antibiotic supplement, the calves showed gains similar to those in the control group. The feeding of aureomycin produced no detrimental effects.

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QUALITY IN ROUGHAGES. I. FACTORS WHICH INFLUENCE HAY COMPOSITION AND QUALITY[1]

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As a follow-up to the preliminary work reported (1) in 1946, a comprehensive long-time project on quality in roughages was organized early in 1947. Results for the 1947, 1948 and 1949 seasons are now available and constitute the basis of this first progress report.

REVIEW OF LITERATURE

The literature on haymaking is voluminous and much of the earlier work is more or less empirical. In many investigations the emphasis was primarily on yield, and previous to about 1920 apparently no extensive studies had been carried on regarding the chemical changes which take place during growth, the curing process and the storage of hay. Although many fodder analyses were made, they dealt almost entirely with the nutrient values of the hays as they were fed out and were not related to the changes which take place in curing and storage. Only a few contributions on this subject will be cited here to give a general picture of the developments in this field to date.

Aside from conventional fodder analyses, most of the chemical studies have centered on carotene. Russell *et al.* (17) drew attention to the effect of the curing process in haymaking on the carotene content of hay, pointing out that strong sunlight and high temperatures are particularly destructive. They stated that as much as 80 per cent of the original carotene of hay may be destroyed in 24 hr. of field-curing. Several later investigators (3, 5, 8, 14) have shown that the longer the curing period, the greater the exposure to sunlight, and the higher the air temperature, the greater usually the carotene loss is in haymaking.

Several studies also have been made on the losses of carotene in storage. Kane *et al.* (11) showed that in alfalfa hay stored below 45° F. the average carotene loss per month was about 3 per cent; at temperatures between 45 and and 66° F. the average loss per month was 6.5 per cent; at temperatures above 66° F. average loss was 21 per cent per month during the first summer of storage and 11 per cent during the second summer. Others (4, 5, 8) also have noted large storage losses of carotene, especially at high temperatures.

A few studies have been made on sugar content of hays and a very few on carbohydrates in general. Dexter (6) showed that in hays dried slowly by unheated air over a 3-day period there was a pronounced loss of sugar and some

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[2] J. Bart and A. F. Spelman did much of the analytical work involved. M. L. Blaisdell, as farm superintendent, was the contact man for field operations and sampling techniques. Acknowledgment also is made to P. P. Keyes and R. C. Church for technical assistance in these operations.

loss of starch. These losses were much greater than those observed in similar samples rapidly dried. He further showed that ordinary field-cured hay had a much higher sugar content than hay dried slowly in the mow over a 3-wk. period.

Eklund (7) showed that the principal factors affecting the carbohydrate composition of hay in Sweden are botanical species, time of harvest and conditions of harvest and storage.

Aside from determination of total and digestible protein in hays cured by different methods, very few studies on proteins in hay have been made. Bartlett *et al.* (3) showed that the biological value of the protein in artificially-dried grass was significantly higher (averages 66.5 *cf.* 58.0) than that of a similar lot of grass cured into hay by natural methods (sun curing). Kiesselbach and Anderson (12) showed that 1 in. of rain was without effect on the protein content of alfalfa hay, but that there was a highly significant positive correlation ($r = 0.72 \pm 0.03$ in 253 samples) between leafiness of hay and its protein content.

The effect of meteorological factors during the growing season on chemical composition of hay at cutting time has not been studied to any extent. The one extensive report noted is that of Hvidsten *et al.* (9) of Norway, who studied the problem in some detail for two contrasting seasons: 1942—a warm, dry season; 1943—a cool, wet season.

Kiesselbach and Anderson (12) report that quality of hay, as determined by leafiness, color and protein content, was not significantly affected by numerous variations in curing practice. Scurti and Mossi (18) showed that nitrogenous matter and fat in the leaves of alfalfa and clover decreased with increasing moisture (artifical rains), but that the losses of nitrogen were concerned not with proteins but with amino acids and organic bases. Cellulose and ash apparently increased with increasing moisture; digestibility was adversely affected.

During the past decade, attention has been focused largely on attempts to improve the curing of hay by shortening the length of time in the field after cutting, notably by mow-curing with or without supplementary heat. The earlier work on mow-curing seems to have involved the use of external heat rather generally, with rather unfavorable results. Recent reports are more favorable to the use of supplementary heat, presumably a reflection of improved methods. Strait (20) reports that "hay produced by heated air was of superior quality in every respect". The hay was leafy, green and free from mold, and the carotene loss was only 18.5 per cent of the orginal content; considerably less, he observes, than could be expected under conditions of field curing. King *et al.* (13) recommended supplemental heat for the mow-curing of baled hay. Even with the bales loosely packed, when unheated air is used the drying is prolonged and most of the carotene is destroyed.

The use of unheated air for mow-curing of hay seems to have been tried first in this country by the T. V. A. in cooperation with the Tennessee Experiment Station. Wylie *et al.* (22) described a method of barn-curing by means of forcing air through hay. Partially cured in the field, the hay was placed in a loft over a system of air ducts through which air from a blower was forced upward. This method has been sufficiently successful so that, with numerous modifications,

it has spread to all the major hay-growing regions of this country. Jennings (10) outlined conditions for success with the method and concluded that results were sufficiently promising to warrant further studies.

Despite all these studies, very little has been done on the composition and nutritive value of hay cured in the mow either with or without heat. Monroe *et al.* (16) in a general review of the problem in 1946, stated that "much more work on the nutrient content, feeding value, and palatability of mow-cured hay needs to be done." In the same year MacDonald (15) stated that "the difficulty of curing hay plants at their most nutritious stage of growth without excessive loss, and the problem of maintaining their high nutritive value during handling and storage is one of the greatest problems facing us today in the field of forage production."

Together with the relative dearth of definite information on changes during curing and storage already noted, these statements illustrate the importance of studies on the subject undertaken at this station beginning in 1947, this article constituting the first progress report.

EXPERIMENTAL

The work has involved the collection and analyses of 192 samples of hay, most of them from the University Farm, in varying stages of the curing process from the green hay as cut to hay ready for storage, and also representing varying intervals in storage from 3 or 4 days to 6 mo. or longer. The results from analysis of 174 of these samples form the basis of this report.

Field samples were taken from either swath or windrow at appropriate intervals over the entire area involved. Stored, loose hay with no other lots stored on top of it was sampled by cutting out with a hay knife several areas approximately 2 ft.^{2} and 1 ft. or more in depth at points uniformly distributed over the surface of the mow. From each of the pockets so formed sub-samples then were obtained by reaching into the mass of hay on all sides of each pocket at points well below the surface and withdrawing a small handful. Access to loose hay buried beneath subsequently stored lots was provided for by the installation of removable panels in the sides of the mow, or through the main air duct for some lots of mow-cured hay. Such lots were sampled by a special hay-sampling augur attached to a bit stock with a 12-in. sweep. This tool is similar to one described by Zink (23). Different lots of hay were kept separate by means of wire matting laid between them. Baled hay was sampled by taking a handful from the center of each of 10 per cent of the total number of bales in any given lot, the sampled bales being chosen from each tier of bales in the lot and from locations varying from tier to tier.

Samples of green hay and of hay in various stages of the curing process (up to the point where they had dried out to approximately 20 per cent of moisture) were brought to the laboratory at once, where moisture and carotene content were determined immediately. The balance of the sample then was rapidly dried in a current of air at about 80° C. to inactivate the plant enzymes, a process which was complete in 0.5 to 1 hr. The dried samples were allowed to

return to room temperature, after which they were ground in a Wiley mill, passed through a 0.5 mm. sieve, bottled and stored for subsequent analyses.

Samples of dry hay were stored for varying periods soon after sampling in a refrigerator at about 40° F. in either friction-top pails or air-tight fiberboard cartons. Subsequent treatment for these was the same as described above for the green or partly dry hays, beginning at the point where they were brought to the laboratory.

Analyses made included routine fodder analyses, total sugars determined as reducing sugar and carotene. Fodder analyses were made according to the official methods of the A.O.A.C.(2); sugar was determined by the Lane and Eynon method, as described in (2); and carotene by the method of Wall and Kelley (21).

DISCUSSION OF RESULTS

(a) *Variations in composition of green hay from year to year.* Considerable variation was noted but the differences were not at all consistent; therefore, it has not been considered worthwhile to report the detailed data.

Values for *r* between the amount of rainfall and the several nutrients in the hays and between amount of bright sunshine and the nutrients were not statistically significant, but those for average air temperature were significant in a number of cases.

Values of *r* for Average Air Temperature *cf.* Various Constituents in the Hays.

	Values of r
Moisture in the fresh sample	−0.81 H.S.*
In the dry matter:	
Protein	−0.40 N.S.
Ether extract	−0.73 H.S.*
Crude fiber	−0.07 N.S.
Total ash	−0.59 S.**
N-free extract	0.50 A.S.**
Sugar	−0.71 S.*
Carotene	−0.34 N.S.

A.S. = approaching significance
N.S. = not significant
* at 1% level.
** at 5% level.

H.S. = highly significant
S. = significant

The highly significant negative correlation for moisture in the green hay is what might be expected; the warmer the weather, the dryer the crop, other things being equal. With one exception (N.F.E.), the correlations are all negative. This is in agreement with the well-established observation that the grasses of this region thrive best in relatively cool weather; it also agrees with the work of Spoehr (19) who showed that high temperatures tend to decrease, while low temperatures tend to increase the monosaccharides.

No apparent correlation existed between amount of rainfall during the growing season and the composition of these hays. Probably the effect of rainfall is much more evident in yield of hay than in its composition; also the number of samples available for comparison in this respect possibly was insufficient to demonstrate relationships that might become apparent with larger numbers.

In general, however, weather conditions during the growing season were without substantial influence on the composition of these hays. Hvidsten *et al.* (9) also observed that the content of total organic matter in hay was not greatly affected by the kind of season (cool and wet *cf.* warm and dry).

(b) *Variations in composition of hays as a result of variable weather conditions during hay harvest.*—Table 1 summarizes the changes in composition of several lots of hay that had been rained on during the curing process in contrast with several other lots that had not been rained on.

The average composition of the several lots of hay was about the same when stored whether they had or had not been rained on. Except for total sugars the greater losses were in those lots that had not been rained on. For carotene, although the relative loss was greater in the hays exposed to rain, the absolute

TABLE 1

Changes in composition of hays rained on and not rained on

	Moisture	Percentages in the dry matter of:						
		Protein	Ether extract	Crude fiber	Total ash	N.F.E.	Total sugars	Carotene
	(%)							(*ppm.*)
8 lots rained on:								
When cut	66.6	11.3	2.6	35.3	6.0	44.8	6.9	149
When stored	27.2	11.2	2.2	36.2	6.1	44.4	6.5	55
Change	−39.4	−0.1	−0.4	+0.9	+0.1	−0.4	−0.4	−94
% change	−59.2	−0.9	−15.4	+2.5	+1.7	−0.9	−5.8	−63.1
12 lots not rained on:								
When cut	71.7	11.3	3.0	34.2	6.6	44.9	6.2	181
When stored	30.5	11.2	2.3	36.1	6.7	43.7	6.3	78
Change	−41.2	−0.1	−0.7	+1.9	+0.1	−1.2	+0.1	−103
% change	−57.5	−0.9	−23.3	+5.6	+1.5	−2.7	+1.6	−56.9

Average amount of rainfall—0.37″. Range 0.05″–1.02″.

loss was 9 ppm. less than in the other group. Fiber shows a greater gain in the lots not rained on, but from the standpoint of nutritive value, this is actually a loss. In fairness it must be admitted that the amounts of rainfall involved were not great, but it should be noted that the results, especially with respect to protein content of hays, are in agreement with those reported by Kiesselbach and Anderson (12) and by Scurti and Mossi (18).

The effect of exposure to bright sunlight during the hay-curing process also has been studied and for this purpose results have been used from the 12 lots of hay reported in the second part of table 1 as not rained on. The periods of exposure to sunlight varied from 11.5 to 33 hr. averaging 20.25 hr.

The general effect of sunlight was to lower the ether extract and the carotene and to increase the fiber, with no significant change in protein, ash, N-free extract or sugar. The downward trend in carotene and the upward trend in fiber with increasing exposure to sunlight have been plotted in figures 1 and 2 by the

method of least squares.[3] Continued exposure to sunlight apparently did these hays more harm than was done to the other eight lots by the moderate amounts of rainfall—at least if maximum amounts of carotene and minimum amounts of fiber are any criterion of nutritive value. Numerous other investigators have

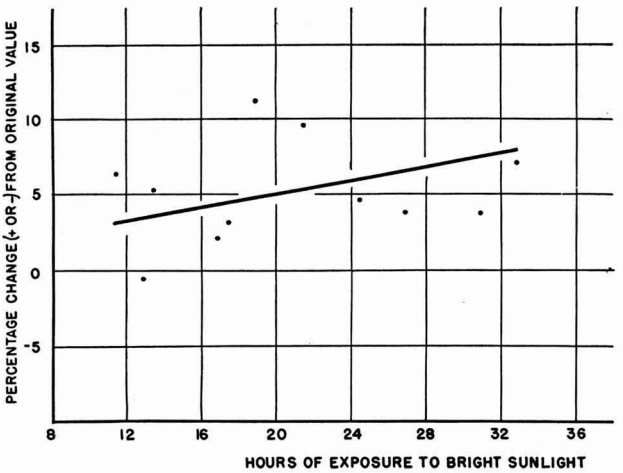

FIG. 1. Change in Crude Fiber Content of Hays With Increasing Exposure to Sunlight.

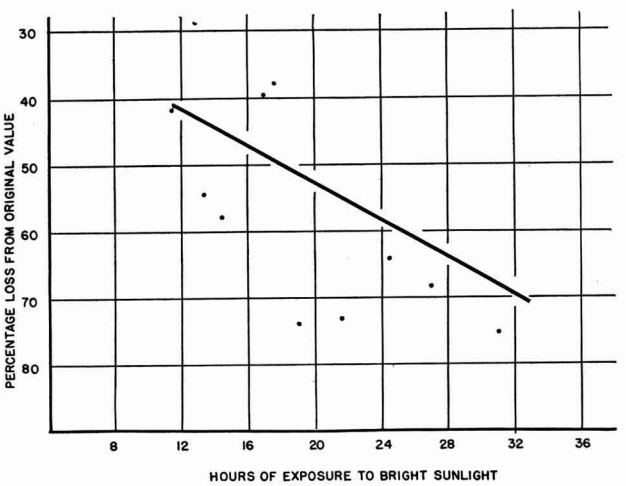

FIG. 2. Decrease in Carotene Content of Hays With Increasing Exposure to Sunlight.

noted the rapid drop in carotene as field-curing of hay progresses, but in the lay mind, at least, this is often attributed to the poor curing conditions associated with wet weather rather than with the action of sunlight.

[3] The values for ether extract do not show a consistent scatter pattern, and therefore the graph for this constituent has not been included.

Possible differences between field-cured and barn-cured hay as a reflection of differing lengths of time in the field after cutting also have been studied; the results are summarized in table 2. The average length of curing time in the

TABLE 2

Effect of the different length of time in the field after cutting, involved in field-curing cf. barn-curing

	Moisture as sampled	Percentages in the dry matter of:						
		Protein	Ether extract	Crude fiber	Total ash	N-free extract	Total sugars	Carotene
	(%)							(*ppm.*)
Average of field-cured hays—11 lots; 64-hr. exposure								
When cut	70.0	11.5	2.8	34.4	6.3	45.0	6.6	181
When stored	24.4	11.3	2.2	36.1	6.5	44.0	6.5	63
Change	−45.6	−0.2	−0.6	+1.7	+0.2	−1.0	−0.1	−118
% change	−65.1	−1.7	−21.4	+4.9	+3.2	−2.2	−1.5	−65.2
Average of barn-cured hays—9 lots; 47-hr. exposure								
When cut	69.3	11.1	2.8	35.0	6.4	44.7	6.3	152
When stored	35.0	11.1	2.3	36.3	6.3	44.0	6.2	78
Change	−34.3	0.0	−0.5	+1.3	−0.1	−0.7	−0.1	−74
% change	−49.5	0.0	−17.9	+3.7	−1.6	−1.6	−1.6	−48.7

field was 64 hr. for those lots completely field-cured and 47 hr. for those where the curing was completed over forced air draft in the barn. Except for carotene content, there was no significant difference in composition at time of storage between field-cured and barn-cured hays. The higher carotene content of the barn-cured hays when stored is doubtless a reflection of the shorter time these remained in the field after cutting (17 hr. less on the average).

(c) *Changes in composition during storage.* Changes taking place during storage have been followed for 28 different lots of hay grown in 1947, 1948 and 1949; samples were taken at varying intervals of time from the time the hays were stored until they were fed out. The over-all picture is summarized in table 3. The significant[4] changes noted are an increase in fiber, and decreases

TABLE 3

Over-all changes in composition of hays in storage

Av. no. days in storage	Moisture as sampled	Protein	Ether extract	Crude fiber	Total ash	N-free extract	Total sugars	Carotene
	(%)							(*ppm.*)
0	30.5	9.7	2.2	36.5	6.0	45.6	6.3	69
192	10.5	8.8	2.3	39.2	6.2	43.5	5.1	13
Percentage change	−65.6	−9.3	+4.3	+7.4	+3.3	−4.6	−19.0	−81.2

in N-free extract, sugar and carotene. The only excessive decrease is that for carotene, over four-fifths of which had been destroyed by the time the several lots of hay were fed out.

[4] The term "significant" as used in this report means "statistically significant."

In an attempt to determine when these changes take place, samples were taken at 4, 8, 32 and 192 days in storage. The results of these periodic analyses are summarized in figure 3.[5] As time in storage elapsed there was: (a) A slow, steady increase in fiber; (b) a decrease of somewhat similar rate and magnitude in N-free extract; (c) a rapid decrease in protein during the first week in storage, which leveled off thereafter to a much slower rate of decrease; (d) a considerable decrease in total sugar during the first week and a somewhat slower decrease up to the end of a month, after which time there appears to have been no further decrease; (e) a very rapid decline in carotene at first followed by less rapid but still substantial losses to the end of the storage period. The close similarity between the curve for carotene and that for moisture shows that destruction of carotene proceeds along with the dehydration process.

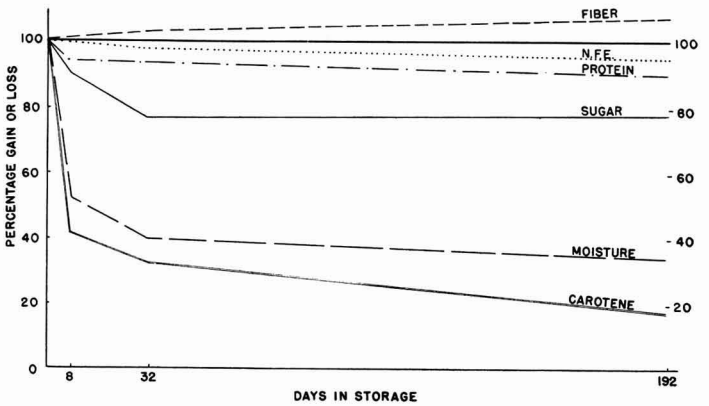

FIG. 3. Progressive Changes in Composition of Hays During Storage

The relative effect of field-curing *cf.* barn-curing on changes during storage also has been studied and results are summarized in table 4. Although the barn-cured hay when stored was slightly but significantly higher in protein, sugar and carotene, by the time the several lots were fed out, the small differences were no longer of any significance. The significantly higher carotene content of barn-cured hay at time of storage was almost completely nullified by the changes taking place in storage.

That there was little practical difference in feeding value between hays cured by these two methods was further confirmed by feeding trials of the hays for milk production conducted with eight cows in the University herd in each of two winter seasons, 1948–49 and 1949–50. The cows—Ayrshires the first year, Holsteins and Guernseys the second—were divided into two groups of four cows each and fed field-cured and barn-cured hays from the same fields by the double reversal system over a period of 120 days in each year. The 120-day period was

[5] Results from those samples taken after 4 days in storage have not been included in the summary. They are not in agreement with the rest of the data, probably due to a somewhat smaller number of samples.

divided into four sub-periods of 30 days each, so that each group of cows received each kind of hay at two different times for 30 days. Milk production for the last 20 days of each period was used as the basis for calculating the results. Since milk yield on the F.C.M. basis per pound of dry matter consumed, was identical for the two kinds of hay, these results need no further comment.

Since it is well known that the presence of moisture facilitates fermentation, a study was made of the possible correlation between moisture content of the hays at the time of storage and the subsequent changes in their composition. For the entire group there was a distinct correlation between moisture when stored and subsequent losses in carotene and in the carbohydrate fraction of the hays. Expressed in another way, the higher the moisture content of the hay, the greater, in general, were the storage losses of these nutrients. Protein and fiber do not appear to have been materially affected.

TABLE 4

Changes in composition during storage as affected by method of curing

Length of time in storage	Moisture as sampled	Protein	Ether extract	Crude fiber	Total ash	N-free extract	Total sugar	Carotene
(*d.*)	(%)							(*ppm.*)
Barn-cured—14 lots								
0	37.0	10.0	2.1	36.0	6.0	45.9	6.4	78
191	10.4	8.6	2.3	39.1	6.1	43.9	5.2	13
Percentage change	−71.9	−14.0	+9.5	+8.6	+1.7	−4.4	−18.7	−83.3
Field-cured—14 lots								
0	23.9	9.4	2.2	37.0	6.0	45.3	6.2	61
193	10.7	9.0	2.3	39.4	6.3	43.0	5.0	12
Percentage change	−55.2	−4.3	+4.5	+6.5	+5.0	−5.1	−19.4	−80.3

Carotene loss in storage definitely was correlated with high moisture content whether the hays were barn-dried or field-cured.

Although there was a significant correlation between moisture content and losses of carbohydrate (N.F.E. and sugar) in the field-cured hays, this correlation was not significant in the barn-cured hays, indicating possibly that the more rapid drying reduced to some extent the degree of fermentation taking place after storage.

A progressive increase in N-free extract and sugar content of the hays grown in successive years, accompanied by a similar progressive decrease in moisture content when stored, in both barn-cured and field-cured hays, is further confirmation of the above observation. Apparently, a better job of curing, especially of barn-curing, was done as the work progressed and experience was gained.

(d) *Miscellaneous observations on unusual conditions to which some lots of hay were subjected.* The isolated cases reported in table 5 have been chosen to illustrate what happens to hays when, for one reason or another, the curing

process has not been carried out properly. Four of the samples came from the University farm, the other two from a private owner. The principal losses are in sugar and carotene, protein remaining remarkably constant. Of particular interest is the contrast between lots A and B and lots C and D in baled storage.

TABLE 5

Some contrasts in composition due to improper handling of hay in storage

Lot	Sampling date	Moisture as sampled	Protein	Ether extract	Crude fiber	N.F.E.	Total ash	Total sugar	Carotene
		(%)							(*ppm.*)
A	Dec. 1/47	8.5	23.7	2.2	28.5	39.6	5.9	2.1	6
B	Dec. 1/47	8.0	18.4	2.2	28.9	43.3	7.2	7.1	65
C	Aug. 12/47	34.6	13.1	2.5	36.6	40.9	6.0	6.5	75
D	Sept. 9/47	16.2	12.6	2.5	38.1	39.6	7.2	2.6	13
E	Aug. 18/48	28.4	15.3	1.7	37.9	37.9	7.2	5.4	64
F	Sept. 29/48	12.0	14.8	2.4	36.2	38.9	7.7	2.7	26

Description and remarks

A. Alfalfa and grass; second cutting; baled and stored on wagon for 1-wk. before being barn-dried; leafy but bleached.

B. Same field as above; third cutting; baled and put over dryer immediately; excellent product; very leafy; color and odor practically as when cut.

C. Alfalfa, red clover and timothy; early bloom stage; second cutting; sampled just before baling; barn dried.

D. Same lot as C four weeks after baling; poor quality, musty, moldy, dusty; inefficient blower, belt slippage.

E. Alfalfa; second cutting; in full bloom; sampled at loading time; beautiful quality but a bit too damp for safe storage.

F. Same lot as E; 5 wk. after baling; markedly musty; had lost all its green color; bleached to a dirty gray brown.

The general conclusion, not only from these isolated cases but also from our other studies, is that it is very easy to spoil good hay by an indifferent job of curing, especially barn-curing, either by storing the hay when too damp or because of inadequate air flow, or both.

(e) *Correlation as a guide to future studies in searching for an index of quality.* In this phase of analysis of results, values for r have been determined for six nutrients in 172 samples of hay. These values are tabulated below:

Between:	r =	*Between:*	r =
Protein and ether extract	0.40*	Ether extract and sugars	0.02 N.S.
Protein and crude fiber	−0.46*	Crude fiber and N-free extract	−0.27*
Protein and N-free extract	−0.60*	Crude fiber and ash	−0.23*
Protein and total ash	0.65*	Crude fiber and sugars	−0.26***
Protein and total sugars	−0.40*	N-free extract and ash	−0.62*
Ether extract and crude fiber	−0.49*	N-free extract and sugars	0.51*
Ether extract and N-free extract	0.19**	Total ash and sugars	−0.39*
Ether extract and ash	0.48*		

N.S. = not significant.
* Highly significant at the 1% level.
** Significant at the 1% level.
*** Significant at the 5% level.

Since the great majority of the values for r (12 out of 15) were highly significant, it would seem that considerable confidence can be placed in them. Fiber is negatively correlated with all the other constituents, which is not surprising

except perhaps in the case of fiber *cf.* ash. A rather clear-cut balance appears between protein and ash on the one hand and N-free extract and sugars on the other hand, with ether extract occupying a middle ground. It should be noted, however, that the correlation between ether extract and sugars was not significant; and between ether extract and N-free extract, significant only at the 5 per cent level.

In such a situation no one constituent, fiber excepted, offers even tentative help as an indicator of possible nutritive value in a hay. If a hay is relatively high in protein and ash, then judging from these relationships, it is somewhat better than an even chance that it will be low in those nutrients which furnish the bulk of its energy; viz., sugars and the other non-lignin fractions of the N-free extract.

Fiber seems to be a rather consistent indicator of value in a negative sense, but its determination is more time-consuming and arbitrary than that of any other of these nutrients, and it is open to the further criticism that bacteria in the rumen are able to digest a considerable portion of the cellulose which makes up the bulk of crude fiber.

In the present state of knowledge, actual feeding trials are the most reliable guide to nutritive value of hays and other roughages. With large animals, however, such trials are expensive, time-consuming, and often inconclusive; therefore, our search for a more readily determined index of roughage quality will be continued.

SUMMARY

Average air temperature during the growing season was negatively correlated with moisture in the green hay and with ether extract, total ash and sugar in the dry matter.

Amounts of rainfall and sunshine during the growing season were without substantial effect on the composition of these hays.

The principal loss during curing of these hays was in carotene; exposure to bright sunlight was more destructive of carotene than was a moderate amount of rain. Protein was substantially unaffected by the curing process, regardless of weather.

Hays in which the curing process was completed by mow drying were significantly higher in carotene at time of storage than those completely cured in the field.

Changes in composition during storage were considerably greater than those which took place in the field after cutting. The significant changes in storage were an increase in fiber, and a decrease in N-free extract, sugar, and carotene, the losses of carotene being very high.

Most of these changes during storage took place in the first month, a large part of them during the first week of storage.

Losses of N-free extract, sugars and carotene during storage were significantly correlated with moisture content of the hays at time of storage. This was true for carotene regardless of method of curing. In barn-dried hays the

correlation of losses in N-free extract and sugars with moisture content was not significant; it was significant for field-dried hays.

Although barn-cured hays had a higher content of carotene than field-cured hays when stored, this advantage had disappeared almost entirely by the time the hays were fed out.

Feeding trials for milk production with the two kinds of hay gave identical results in fat-corrected milk yield per pound of dry matter consumed.

Some illustrations are given of how easily good hay can be spoiled by an indifferent job of curing, especially barn-curing.

None of the constituents determined, with the possible exception of fiber, offers any promise as an indicator of the nutritive value of hay. The search for such an indicator will be continued.

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2-THIOBARBITURIC ACID AS A REAGENT FOR DETECTING MILK FAT OXIDATION[1]

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Kohn and Liversedge (3) observed that various animal tissues after incubation under aerobic conditions, produce compound(s) which give a color reaction when heated with thiobarbituric acid (TBA). Although they did not demonstrate the nature of the color producing compound(s), their research established the absorption spectrum of the color complex and certain chemical characteristics of the color reaction. Research by Bernheim *et al.* (2) revealed that the color-producing materials in various animal tissues are lipid and, more specifically, that egg lecithin or fatty acids isolated from it, brain lecithin, fatty acids from linseed oil, cephalin and brain protein-lecithin complex give colors with TBA after aerobic incubation. The absorption spectra of the colors obtained from these materials were shown to be identical with that described by Kohn and Liversedge and that obtained with linolenic acid. More recently Wilbur *et al.* (5) have further explored the TBA color reaction with regard to certain sugars and aldehydes, as well as the oxidation products of linolenic and certain other unsaturated fatty acids. These studies revealed no specific compound which gives a color spectrum identical with those obtained from oxidized lipid materials or aerobically incubated animal tissues.

A knowledge of the compound(s) which produce the typical color on heating with TBA not only might clarify certain aspects of fat oxidation, but also could give some insight into the biological utilization of unsaturated fatty acids in animal tissues. The above-mentioned studies have indicated that the TBA reagent is particularly sensitive in measuring photo-chemical, biological or autoxidation of lipid materials. The possibility that this reagent might be applied usefully in the measurement of milk fat oxidation therefore is quite evident. This paper is concerned with a fundamental investigation of the TBA test and its application to the measurement of milk fat oxidation.

EXPERIMENTAL

The color reaction of TBA with milk fat. It was of primary interest to ascertain whether milk fat would respond to the TBA test. TBA (Eastman) reagent was prepared, as directed by Kohn and Liversedge (3), and the test applied to dry milk fat samples of various ages prepared according to conventional procedures from fresh sweet cream. Quantities of melted fat (1.1 ml.) were placed in 5-in. test tubes and, successively, 2.5 ml. of water, 2 ml. of TBA reagent, 1 ml. of 20 per cent trichloroacetic acid and a boiling chip were added, after which

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the tubes were placed in a boiling water bath for 10 min. Fresh fats gave essentially no color in the test, whereas aged fats invariably gave a characteristic red color to the aqueous phase. The amount of color produced in the test appeared to be positively correlated with the age of the fat.

Compounds producing the color. The chemical identity of the lipid oxidation product(s) responsible for the TBA color reaction is not known. Bernheim *et al.* (2) have isolated and analyzed the pigment. Their data indicate that a three-carbon compound reacts with the reagent in producing the color. Other work (3) has shown that the responsible compound(s) are carbonyl in nature, since semicarbazide or phenylhydrazine will block the reaction. The possible importance of peroxide compounds also has been suggested in this connection (5).

Wilbur *et al.* (5) have submitted a number of aldehydes and sugars to the TBA test. Of these, only glyoxylic acid was observed to give a red color and spectral analysis of this color revealed maxima at 525 and 550 mμ rather than a single peak at 532 mμ, characteristic of the color from oxidized lipid materials.

In this study, a number of compounds in addition to those investigated by Wilbur *et al.* (5) were subjected to the TBA test. These included epichlorohydrin, glycidol (epihydrin alcohol), the oxidation (H_2O_2) products of glycidol, glyoxal, methyl glyoxal, malonic dialdehyde diacetal, malonic dialdehyde, diethoxytetrahydrofuran, diacetone alcohol, acetyl acetone, acetonyl acetone, 1,4-dioxane, trimethylene glycol and the oxidation (both H_2O_2 and $KMnO_4$) products of trimethylene glycol. The compounds were tested in the same manner as that described for milk fat. Five- to ten-mg. quantities were placed in test tubes together with the reagents; the tubes and contents then were heated in a boiling water bath for 10 min., after which color was noted. Of the compounds tested, only glyoxal, malonic dialdehyde, its diacetal and the oxidation products of trimethylene glycol gave the characteristic red color.

The spectral characteristics of the color complexes obtained from malonic dialdehyde and from oxidized milk fat on heating with TBA were determined in a Beckman model DU spectrophotometer. The sample of milk fat employed had been stored in a brown glass container for 3 mo. in the laboratory. The color density in the malonic dialdehyde sample was adjusted to approximate that of the fat sample. The data for these two systems are presented in figure 1. It is evident from these data that the colors produced with malonic dialdehyde and with oxidized milk fat are identical. The absorption maxima at 532 mμ and the shape of the transmission curves for these two color systems are essentially the same as those for oxidized methyl linolenate (5), oxidized phospholipids (2) and aerobically incubated animal tissues (3, 5). The color produced by malonic dialdehyde diacetal and the oxidation products of trimethylene glycol logically may be ascribed to malonic dialdehyde. In the case of the former, liberation of free dialdehyde would be promoted by the acidic conditions of the reaction mixture. Oxidation of trimethylene glycol also would be expected to yield small quantities of malonic dialdehyde. Spectral analysis of the glyoxal-TBA color complex was made also. In this case, no peak was observed at 532 mμ; rather, maxima at 525 and 550 mμ were noted.

General observations on the chemistry of the color reaction. Concentrations of malonic dialdehyde diluted to the extreme (< 1 ppm.) give distinctly positive TBA tests. Moreover, it is quite plausible that this compound results from the oxidation of unsaturated fatty acids. However, in the light of certain observations made in this laboratory, malonic dialdehyde as such could account for only part of the color produced on heating oxidized milk fat. Exhaustive extraction of oxidized milk fat with hot water was observed to remove only part of the substances responsible for color in the TBA test. Under these conditions malonic dialdehyde should be extracted readily. As shown in figure 2, even freshly prepared milk fat will give some color when heated with TBA, and the color density increases in proportion to the length of time the fat is refluxed with the reagents. The data in figure 2 were obtained by periodically removing a 5-ml. sample of the

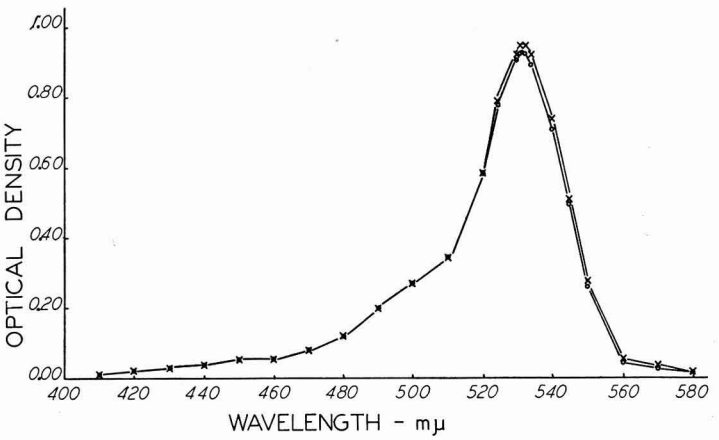

FIG. 1. The absorption spectra of the colors produced with TBA by malonic dialdehyde (O) and oxidized milk fat (X).

aqueous phase of a reflux mixture of 40 g. of fat, 100 ml. of water, 80 ml. of TBA reagent and 40 ml. of 20 per cent trichloroacetic acid, then measuring the color of the sample in a Klett-Summerson colorimeter with no. 52 filter. The milk fat samples employed in the experiment were from the same lot of fat. The aged sample was held in an Erlenmeyer flask exposed to laboratory light during a 24-day storage period. The data amply reflect the effects of these storage conditions in increasing the amount of color produced in the TBA test.

Measurement of milk fat oxidation with the TBA reagent. A considerable amount of experimentation has evolved the following procedure for measuring milk fat oxidation with TBA. Melted milk fat (3.00 g.) is weighed into a 50-ml. wide-mouth Florence flask; 7.5 ml. of distilled water, 6.0 ml. of TBA reagent, 3.0 ml. of 20 per cent trichloroacetic acid and a few boiling chips are added. The flask then is fitted to a condenser and gently refluxed for 10 min., time of reflux being measured from the time at which the mixture commences boiling. A 5- to 10-ml. sample of the aqueous layer is transferred to a calibrated colorimeter

tube. Any existing turbidity can be eliminated by adding 2 ml. of petroleum ether to the sample in the colorimeter tube, shaking the tube contents for a few minutes and then centrifuging the sample (2,500 rpm. for 5 min.). Color density of the samples may be determined in a Klett-Summerson photoelectric colorimeter with either no. 52 or 54 filter. A distilled water blank may be used to zero the colorimeter. Blank values on the reagents were obtained periodically and invariably gave readings of six units, very nearly the same as distilled water.

Although data secured to date using the above method are of a preliminary nature, it would seem desirable to give a resumé of them. Freshly prepared samples of milk fat from fresh, high quality sweet cream give colorimeter values

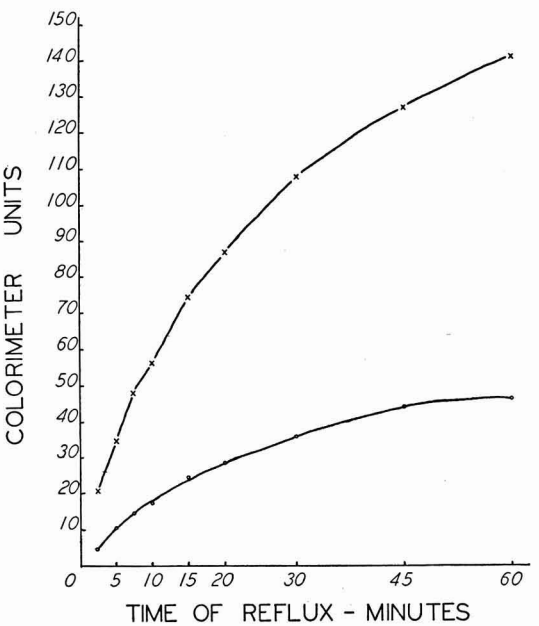

FIG. 2. Color development resulting from the heating of fresh (O) and 24 day old (X) dry milk fat with TBA reagent.

of 12 to 16. This amount of color is a barely perceptible pink. Depending on storage conditions, the color intensity increases with age of the fat, even under refrigeration (4° C.) in the dark and with a minimum of oxygen present. For 2-mo. refrigerated storage an average increase of four to five units has been observed. For comparable fat samples, stored at room temperature (22 to 25° C.) and at 40° C., increases in TBA values of 6–8 and 12–14 colorimeter units, respectively, have been noted. Much more drastic increases in color values result if the sample is exposed to light, air or metallic contamination. When fat samples are stored under optimum conditions at 22 to 25° C., first changes in flavor of the fat become evident when color values of 20 to 30 are reached. Flavor at this point is mainly of the "heated," "nutty" type. In the region of 30 to 40 color

units a definite off-flavor is evident in the fat which appears to be a combination of strong "heated" and slight "oxidized." Above 40 units increasing degrees of "oxidized" flavor become evident.

DISCUSSION

It has been observed that both malonic dialdehyde and oxidized milk fat give a red color when heated with TBA. Spectral analysis of the two colors has indicated that they are identical (fig. 1) and further, that they closely resemble colors obtained under similar conditions from a wide variety of oxidized lipid materials (2, 5).

The potential significance of malonic dialdehyde in this regard may be of more than passing interest since a number of studies employing thiobarbituric acid have dealt with biological oxidation (*in vitro*) of unsaturated fatty acids in animal tissues (1, 2, 3, 4, 5).

Malonic dialdehyde cannot be solely responsible for the color reaction between oxidized milk fat and TBA reagent, since the color-producing compounds of the oxidized fat are only partially removed by exhaustive extraction with hot water. However, the colors from the two materials appear the same and further Bernheim *et al.* (2) have indicated that a three-carbon compound containing oxygen adds to the reagent in the formation of the pigment. Possibly, where the malonic dialdehyde does not exist in free form, TBA may react with certain groupings in the oxidized lipid and effect cleavage of the carbon chain to obtain the structure necessary for completing the pigment. The relative insolubility of the color in fats and non-polar solvents also would indicate that chain cleavage occurs and that no appreciable fatty residue is associated with the color compound. The above contention would be considerably strengthened by demonstration of malonic dialdehyde as an oxidation product of lipids. In any event, other lipid oxidation products, in addition to malonic dialdehyde, may give rise to the color with TBA. An interesting coincidence is that malonic dialdehyde gives a very pronounced Kreis test, a classic color reaction of oxidized lipids.

This study suggests that TBA is a particularly sensitive reagent for detecting milk fat oxidation. Such determination as iodine number and Kreis color reaction of milk fat frequently are unsatisfactory due to the fact that milk fat usually has deteriorated beyond the point of being palatable by the time these tests show significant change. The TBA reagent appears to overcome this objection. For example, samples of milk fat stored in this laboratory have progressively reached TBA values of 200 colorimeter units, a decided pink color. Up to this point, these samples consistently demonstrated no Kreis test, yet the presence of a pronounced degree of oxidized flavor in the fats was obvious.

The procedure for using the reagent with milk fat as presented herein will, in all probability, bear further refinement. The possible use of data from the TBA test to support organoleptic data, to aid in grading certain dairy products and to enable some estimation of their storage life would justify further evaluation of the test. In addition to dry milk fat and butter, any dried food product

containing an appreciable amount of extractable fat could be conveniently submitted to the test.

SUMMARY

Malonic dialdehyde has been shown to yield a red color when heated with 2-thiobarbituric acid (TBA) reagent. Spectral analysis of this color has revealed it to be identical with that obtained similarly from oxidized milk fat and to closely resemble colors secured in like manner from a number of oxidized lipid materials and animal tissues containing unsaturated fatty acids. Thus, malonic dialdehyde may be a compound of significance in both food fat rancidification and the biological oxidation of unsaturated fatty acids.

A preliminary study concerning the use of TBA to measure oxidative deterioration in pure milk fat has indicated that this reagent is more sensitive than conventional tests such as iodine value and the Kreis test. A procedure for empirical measurement of milk fat oxidation with the TBA reagent is presented. It seems probable that the TBA test could be used advantageously in measuring oxidative deterioration in a wide variety of fats and fat-containing foods.

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AUREOMYCIN CONCENTRATION IN MILK FOLLOWING INTRAMAMMARY INFUSION AND ITS EFFECT ON STARTER ACTIVITY[1]

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The use of antibiotics as a means of eliminating infectious mastitis in dairy herds is well established. Persistent high post-treatment levels of the antibiotics increase their therapeutic value, but the presence of the antibiotics in the milk may prohibit use of the milk for cultured dairy products. The inhibitory effect of penicillin and certain other antibiotics on acid production has been reported by Hunter (7, 8), Katznelson and Hood (10), Kastli (13), Jorgensen (9) and Krienke (11). The use of aureomycin in treating certain forms of mastitis has been reported by Bell and Jordan (1), Easterbrooks (5), McCullock *et al.* (14) and Packer (15). The inhibitory effect of aureomycin on starter activity has been reported by some workers. Krienke (12) reports that the milk of three of five cows treated contained sufficient aureomycin twelve milkings after treatment to retard acid production. Hansen *et al.* (6) report that penicillin, streptomycin, aureomycin, sulfanilamide and sulfamerazine, when used as a treatment for mastitis, are given off in the milk in sufficient amounts to restrict the growth of lactic acid bacteria, and that, if as little as 1 per cent of the milk is from a treated quarter, acid production will be restricted for from four to six post-treatment milkings. Previous experience by the authors indicated that the presence of aureomycin in post-treatment milk samples rendered such milk unsuitable for use in preparing cultured milk products. This study was undertaken to determine the aureomycin concentrations in milk following intramammary infusion and the effect of the antibiotic on starter activity.

EXPERIMENTAL

Ten Holstein cows that were producing more than 30 lb. of milk daily and that were free from any evidence of mastitis or other infectious disease were selected for intramammary infusion. Each quarter was infused with 200 mg. of aureomycin HCl in 7.5 g. ointment base[4] after milking on the first day of the test. Composite milk samples were taken from each cow at the regular milking time under asceptic conditions and either used immediately or stored at refrigerator temperature until examined the following day.

The assay method, in general, followed the procedures outlined by Dornbush and Pelcak (4) and McCullock *et al.* (14). The milk samples were acidified by

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[3] Department of Dairy Husbandry.

[4] The aureomycin used for the infusions and assay and the test organism were furnished through the courtesy of the Lederle Laboratories Div., American Cyanamid Co.

adding 0.1 ml. of 7*N* H_2SO_4 per milliliter of milk. After mixing, the samples were warmed for a few minutes in a 37.5° C. water bath and then centrifuged at 2,500 rpm. for 15 min. Initial dilutions of the clear supernatants were made in penicillin assay broth (Difco) when necessary. Serial 1:1 dilutions of the whey then were made with penicillin assay broth and 0.5 ml. of each dilution were placed in a tube. An aureomycin stock solution was diluted to give a solution containing 0.2 γ per milliliter. A series of standard tubes was prepared by placing 0.5 ml. of the dilute aureomycin solution in the first tube and by serially diluting a second 0.5-ml. portion through five additional tubes with 0.5 ml. of broth. A 1 per cent suspension of an 18- to 24-hr. broth culture of *Bacillus cereus* no. 5 was made with broth and 1.5 ml. of the culture suspension were added to each of the tubes. All tubes then were incubated at 37.5° C. for 4 hr. and the end point taken as the final clear tube of the series.

For starter activity test by method *A*, 100 ml. of the milk sample were placed in a 250 ml. sterile flask, autoclaved at 15 lb. pressure for 10 min. and cooled at 21° C. The sample then was inoculated with 1 ml. of commercial starter[5] and incubated for 16 hr. at 21° C. Nine ml. of the thoroughly mixed incubated sample were titrated with 0.1 *N* NaOH to a faint end point, using phenolphthalein as the indicator. The data are recorded as the percent titratable acidity.

For starter activity test by method *B*, 10 ml. of the milk sample were placed in a sterile test tube. The temperature was adjusted to 37.5° C. and 0.9 ml. of starter added. The inoculated sample was incubated at 37.5° C. for 3.5 hr. The contents of each tube, together with 5 ml. of distilled water used to rinse the tube, were titrated with 0.1 *N* NaOH to a faint pink color, using phenolphthalein as the indicator. The results are reported as the per cent titratable acidity.

For starter activity test by method *C*, the same procedure was used as for *B*, except the milk samples were autoclaved at 15 lb. pressure for 10 min. prior to the adjustment of the temperature to 37.5° C. and the inoculation.

RESULTS AND DISCUSSION

The aureomycin levels obtained in milk following intramammary infusion of 200 mg. per quarter are shown in table 1. Considerable variation in the amounts per milliliter of milk existed from one sample to another at any one sampling. This variation and the levels obtained are comparable with the results obtained by other investigators. A steady decline is observed in the amount of aureomycin found in the milk, but at the end of 72 hr. detectable amounts still are present.

Aureomycin deteriorates under certain conditions (3) and aureomycin HCl is reported to decompose at temperatures above 210° C. (2). The inhibitory effect of the aureomycin in the autoclaved milk samples as determined by method *A* is shown in table 2 and figure 1. These data indicate that under the conditions of the experiment, autoclaving at 15 lb. pressure for 10 min. did not destroy all the aureomycin when it was present in the higher concentrations. Inhibition

[5] The starter used was a commercial buttermilk starter furnished through the courtesy of the Dairy Laboratories, Philadelphia.

of the starter by the milk obtained 12 hr. after intramammary infusion was marked and considerable suppression of activity was noted by the 24- and 36-hr. samples. The aureomycin in the samples obtained at 60 hr. (1.83 γ per ml. before autoclaving) had some inhibitory effect, but the starter activity at this time is approaching normal.

TABLE 1

Aureomycin content of milk following intramammary infusion of 200 mg. per quarter

Cow no.	Prior to infusion[a]	Micrograms per milliliter at: Hours after infusion					
		12	24	36	48	60	72
Control	0	0	0	0	0	0	0
1	0	20	5	8	2	1	1
2	0	10	5	4	1	0.25	0.125
3	0	20	10	8	2	1	0.5
4	0	.....	20	10	4	2	1
5	0	.....	10	4	1	1	0.5
6	0	.....	20	10	4	2	1
7	0	.....	20	5	2	2	1
8	0	20	5	1.25	1.15	1.0	0.5
9	0	40	10	2.5	2.5	4	0.5
10	0	10	5	1.25	2.5	2	0.5
Mean	0	20	11	5.4	2.22	1.83	0.66

[a] Average of 2–4 samples prior to treatment.

The effect of aureomycin on starter activity as measured by the short methods (methods *B* and *C*) is shown in table 2 and figure 2. Milk samples obtained up to 72 hr. after udder infusion and not subjected to 15 lb. pressure for 10 min. (method *B*) showed marked inhibition of the starter. The persistence of the inhibitory effect shown by the raw milk as compared to the autoclaved milk reveals the partial destruction of aureomycin by the sterilizing process. The samples obtained at 12 and 24 hr. after infusion and subjected to 15 lb. pressure

TABLE 2

Inhibitory effect of aureomycin on starter activity

Method	Prior to infusion[b]	Percent titratable acidity[a] at: Hours after infusion					
		12	24	36	48	60	72
A	0.88	0.36	0.43	0.51	0.66	0.83	0.87
B	0.38	0.23	0.25	0.26	0.26	0.27	0.29
C	0.42	0.27	0.33	0.40	0.41	0.43	0.46

[a] Average of 10 samples.
[b] Average of 2–4 samples prior to treatment.

for 10 min. (method *C*) inhibited the starter to a marked degree, but the sample taken 36 hr. after infusion had no effect, although the mean aureomycin concentration in the non-autoclaved samples was 5.4 γ per ml. In addition to the destruction by the sterilization process, there undoubtedly is additional deterioration during the incubation period at 37.5° C.

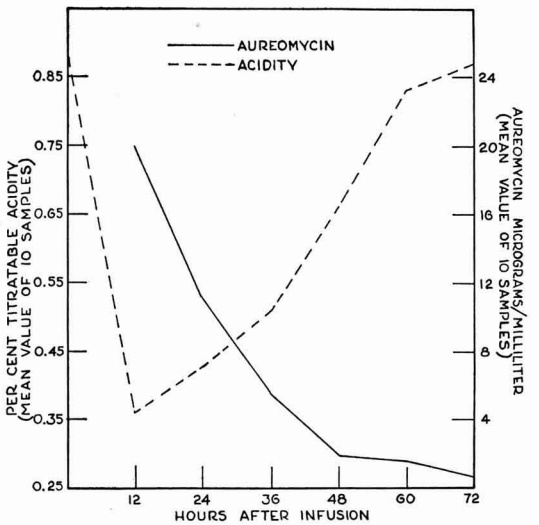

FIG. 1. Effect of aureomycin on starter activity in autoclaved milk (method *A*) held 16 hr. at 21° C. after inoculation.

SUMMARY

The aureomycin content in the samples collected up to 48 hr. after infusion seriously interfered with the starter activity when the inoculated milk was held at 21° C. for 16 hr., even though the milk was autoclaved. The antibiotic level in the samples collected up to 24 hr. after infusion suppressed the starter when the inoculated sample was held for 3.5 hr. at 37.5° C., although the sample had

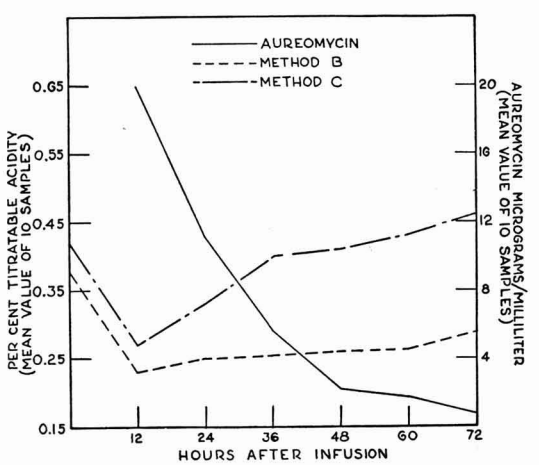

FIG. 2. Effect of aureomycin on starter activity in non-autoclaved (method *B*) and autoclaved milk (method *C*) held at 37.5° C. for 3.5 hr. after inoculation.

been autoclaved prior to inoculation. Marked inhibitory effect was obtained up to 72 hr. after infusion when the raw milk was inoculated directly and held for 3.5 hr. at 37.5° C.

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DETECTION OF ADULTERATION OF MILK BY LACTOMETRIC AND FREEZING POINT METHODS[1, 2]

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The price of milk has been favorable during and following World War II. As a result of this and the fact that milk is bought on a dual standard, *viz.* fat and skimmilk, watering has been practiced.

Watering of milk has been encountered in Iowa. At the time this problem was initiated, cryoscopes were not available. Certain milk buyers in Iowa were using the lactometer to determine total solids in milk and were estimating added water by the limiting formula:

$$\text{I. } \%\text{ added } H_2O = \frac{SS_1 - SS_2}{SS_1} \cdot 100$$

In this formula SS_1 was taken as the legal minimum serum solids (8.5 per cent in Iowa) and SS_2 was the solids percentage found by the lactometric procedure.

It has been shown (11) that, in general, the lactometric formulae and procedures are not particularly accurate for determining total solids in watered milk. The procedure, in which the milk is heated to 45° C. to melt the fat, cooled to 30° C. and the lactometer reading made at the latter temperature, is more accurate than the one in which the lactometer reading is taken at 15.5° C. This is particularly true if the modified Herrington formula is used to calculate the total solids from the lactometer reading of the milk.

It was considered that there was a possibility of increasing the accuracy of formula I, if SS_1 could be estimated in a manner that would approximate the serum solids of the unadulterated milk. A first approximation was made by determining the Babcock fat test of the milk and calculating the serum solids from the fat test by means of the Jacobson regression (7):

$$\text{II. } \%\text{ SS} = 0.4\text{ F} + 7.07.$$

The serum solids value, so calculated, was employed as SS_1 in equation I.

Two difficulties were anticipated with such a procedure: (a) Errors would be introduced as a result of variation (c.f., breed) from the regression and (b) the value of SS_1 would be low because of the fact that the fat test of the watered milk would be lower than that of the sample before adulteration. The first of these errors would be inherent and could not be corrected; it would, in the long run, determine whether or not the method would have any value in determining added water. The second error should introduce a constant deviation for each

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per cent added water and therefore should be subject to correction, if the serum solids contents of the watered milks were determined accurately.

The objective of this study was to check the general procedure indicated above, using the several lactometric methods and formulae previously studied (11), as means of determining total solids in watered milk, in order to determine whether or not the proposed method had practical value.

EXPERIMENTAL PROCEDURE

Lactometric methods were conducted as previously described (11). Babcock fat tests of the milk were determined according to the directions of the A.O.A.C. (2, p. 312–316). Total solids were determined by the Mojonnier method (9, p. 122–126) for use with the Elsdon and Stubbs cryoscopic equation. Freezing points were obtained with a Hortvet cryoscope.

The percentages of added water were calculated by the formulae of the A.O.A.C. (2) and of Elsdon and Stubbs (5). Two average freezing points were employed in the calculations: – 0.550° C. (A.O.A.C., 2) and – 0.544° C. (Elsdon and Stubbs, 4). Likewise, calculations were made using the limiting freezing point (– 0.530° C.) suggested by Stubbs and Elsdon (6).

The milk samples employed in establishing the lactometric procedures were the same ones described previously (11). They included Holstein, Jersey, Guernsey, Brown Swiss and Ayrshire milks. These different breeds were employed to introduce as much variation in milk composition as possible.

The milks that were employed in preparing the "unknown" samples were obtained in part from the College herd, in part from milk patrons of the College Dairy. Whenever the milks were from the College herd, they represented a 24-hr. composite from the animals selected; those from milk patrons were a composite from a single milking. The latter samples were collected under the supervision of a member of the Dairy Industry staff to insure no accidental addition of water.

The samples from the College herd included milks from the same breeds of cattle employed when the lactometric procedures were studied. Six samples were from the College herd, six from milk patrons. Four "unknown" samples were prepared from each sample of milk. All samples that were watered had the water added on a percentage by weight basis; the "skimmed" samples were diluted with their own skimmilks to a desired fat reduction.

RESULTS

Determination of added water when SS_2 was evaluated by the usual lactometric procedures and formulae. The accuracy with which the percentage added water can be calculated by the use of formulae I and II and a specific lactometric procedure is indicated by the data of fig. 1. These data are presented as the differences between the percentages of water calculated and those added. They indicate that no one of the seven formulae or the three lactometric procedures yielded results that were satisfactory. Babcock formula II (3), when used with milks tempered to 15.5° C., yielded results that most closely checked

the amounts of water actually added. The percentages added water calculated generally were low; this would have been expected because the serum solids calculated by these formulae are high (11). Consequently, the values for $(SS_1 - SS_2)$ would be too small. The differences between the percentages of water added and those found became greater for all methods, the greater the amount of added water. This increase in error was much greater with the lactometric formulae which were designed for use at 15.5° C. (regardless of previous treatment of the milk) than with those designed for use at 30° C. (after the milks had been heated to 45° C.).

Determination of added water when SS_2 was evaluated by the modified Herrington formula (11):

$$\text{III.} \quad \% \ SS_2 = \left[1.2537 \ F + \frac{268 \ (L + 3.0)}{L + 1000} - 0.15.\right] - F$$

These data are presented in fig. 2. They indicate that: (a) The mean percent-

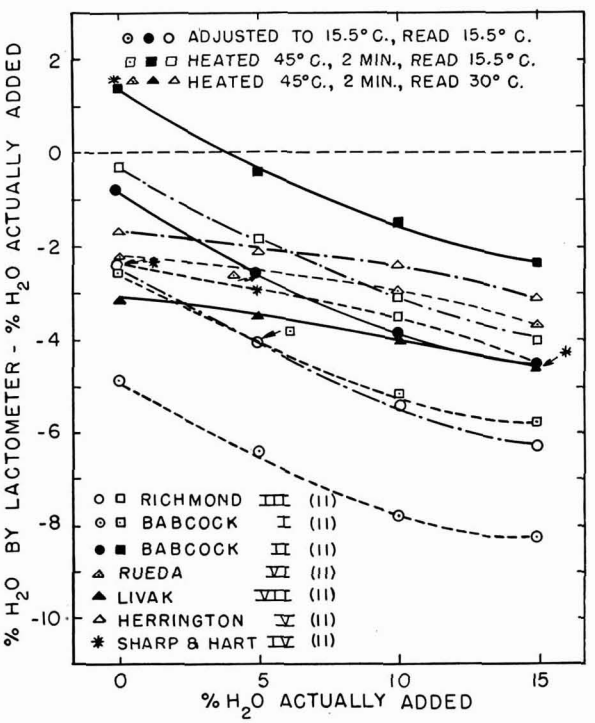

FIG. 1. Algebraic means of errors encountered when the percentage water added to milk was calculated by the formula $\frac{SS_1 - SS_2}{SS_1} \cdot 100$; SS_1 was estimated from the Babcock fat test of the sample by Jacobson's regression ($\%$ SS = 0.4 F + 7.07) (7) and SS_2 was calculated for the sample by the lactometric procedure indicated.

age added water calculated for normal milk is 0.2 per cent; (b) the percentage water calculated decreases linearly as the actual amount of added water increases; and (c) the decrease in amount of calculated water is 0.1 per cent for each per cent water actually added. This is the type of variation that was suggested would result from errors in estimating SS_1 from the fat percentages of the watered milks by use of the Jacobson regression (formula II).

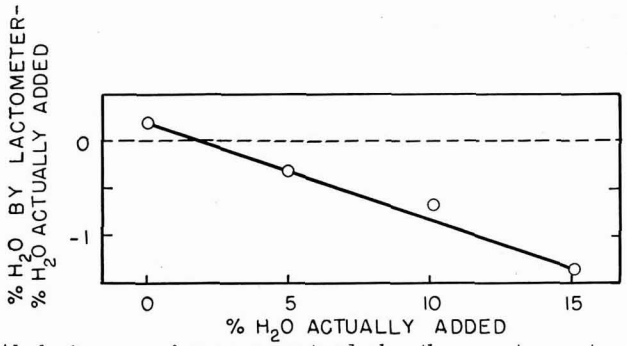

FIG. 2. Algebraic means of errors encountered when the percentage water added to milk was calculated by the formula $\frac{SS_1 - SS_2}{SS_1} \cdot 100$; SS_1 was estimated from the Babcock fat test of the sample by Jacobson's regression ($\%\ SS = 0.4\ F + 7.07$) (7) and SS_2 was calculated for the sample by the modified Herrington formula (11, VIII).

On the basis of these data it was considered that equation I, if employed with equation III, could be modified as follows to yield reasonably accurate mean values for the milks that had been studied:

$$\%\ \text{added}\ H_2O = \left[\frac{SS_1 - SS_2}{SS_1} \cdot 100\right] - 0.2 + \left[0.1\ (\%\ \text{added}\ H_2O)\right]$$

This formula can be simplified as follows:

$$\text{IV.}\quad \%\ \text{added}\ H_2O = 110.89 - 111.11 \cdot \frac{SS_2}{SS_1}.$$

The estimation of the percentage added water by formulae II (for SS_1), III (for SS_2) and IV (for per cent H_2O), together with the Babcock fat test of the milk will be referred to, subsequently, as the "modified lactometric method."

Determination of added water by the modified lactometric method. The data for all normal and watered samples (16 runs) presented in fig. 2, were calculated in accordance with the modified lactometric method. The summary of these data is presented in table 1.

The regression equation relating the water estimated by the modified lactometric procedure and that actually added is:

$\%\ H_2O$, lactom. $= 1.0046\ (\%\ H_2O\ \text{added}) + 0.043$.

The standard error of estimate is 2.181; the correlation coefficient is 0.9360. The close correlation between the water added and that estimated indicates that equation IV compensates for the error in calculating SS_1, which error results

from the fact that the fat test used in equation II is low as a result of the water added. The wide range in fiducial intervals is caused by variation in milk composition, *i.e.*, lack of coincidence between the composition of milks of different breeds, etc. This results in a deviation from the relationship between fat and serum solids that is indicated by the Jacobson regression formula (II). This result is in agreement with observations made previously by Provan (10) and by other workers.

A study of the data indicated that the modified lactometric procedure would be of practical value if properly interpreted. It was considered that, if a single determination were made, watering could be detected with sufficient accuracy for most cases that would be encountered if the following emperical diagnostic procedure, that seems to be justified by the data, were employed: If the percentage added water calculated is: (a) Less than -4.0%, the sample is skimmed, (b) between -4.0 and -1.5%, the sample is suspected as skimmed, (c) between

TABLE 1

Means and fiducial limits[a] of the percentages added water, estimated by the modified lactometric method[b]: at different levels of watering[c]

Prob. level used	Normal milk	Normal milk +5% H_2O	Normal milk +10% H_2O	Normal milk +15% H_2O
95%	0.146 ± 4.813	5.0 ± 4.835	10.181 ± 4.776	14.986 ± 3.604
99%	0.146 ± 6.657	5.0 ± 6.687	10.181 ± 6.604	14.986 ± 4.983

[a] For a single determination, assuming that the sample variances are equal to the population variances.

[b] % added $H_2O = 110.89 - 111.11 \frac{SS_2}{SS_1}$; $SS_1 = 0.4F + 7.07$, (7); $SS_2 = \left[1.2537F + \frac{268\ (L + 3.0)}{L + 1000} - 0.15\right] - F$.

[c] H_2O added as % by weight.

-1.5 and 2.0%, the sample is considered normal, (d) between 2.0 and 5.0%, the sample is suspected as watered and (e) greater than 5.0%, the sample is considered watered. On this basis, 48 "unknown" samples were investigated. These were presented to the analyst as numbered samples with no further identification. The Babcock fat tests and lactometer readings were obtained after samples had been heated to 45° C., held 2 min. and cooled to 30° C. The results that were obtained are presented in table 2.

These data indicate that, in so far as detection of added water in milk is concerned, the modified lactometric method would serve as a rapid sorting method for the College milk supply. With the diagnostic procedure indicated, one could be fairly certain that a milk sample was watered if it were so indicated, since the watered samples in table 2 range from 4 to 15 per cent added water. If the sample were "suspected as watered" it would be advisable to follow this patron's milk during several days and have it checked by the freezing point method if it continued to be indicated as suspected.

Determination of added water by two formulae for use with the freezing point

of the milk examined. Elsdon and Stubbs (5) presented data which indicate that the A.O.A.C. formula (2) for calculating the quantity of water added to milk is in error because of the fact that it does not take into account the variation in water content of the milk resulting from the adulteration. The two formulae in question are:

$$\text{V. } \%\text{ added } H_2O = \frac{T - T_1}{T} \cdot 100 \qquad \text{(A.O.A.C., 2, p. 312–316) and}$$

$$\text{VI. } \%\text{ added } H_2O = \frac{T - T_1}{T} \cdot (100 - \text{T.S.}) \qquad \text{(Elsdon and Stubbs, 5)}$$

in which T is the average freezing point depression for normal milk, T_1 the freezing point depression for the sample under investigation and *T.S.* is the percentage total solids in the sample under observation.

TABLE 2

Results obtained by the modified lactometric method[a, b] *with 48 milk samples*

	Skimmed samples				Normal samples			Watered samples	
	S.	S.S.	N	S.W.	S.S.	N	S.W.	S.W.	W.
Diagnosis[c]	1	4	2	2	1	4	1	1	32
Actual	9					6			33

[a] A single lactometer and duplicate Babcock fat test were employed.

[b] $\%$ added $H_2O = 110.89 - 111.11 \cdot \frac{SS_2}{SS_1}$; $SS_1 = 0.4F + 7.07$ (7); $SS_2 = \left[1.2537\ F + \frac{268\ (L + 3.0)}{L + 1000} - 0.15\right] - F.$

[c] S = skimmed; S.S. = suspected as skimmed; N = normal; S.W. = suspected as watered; W = watered.

The average freezing point depression recommended by the A.O.A.C. is 0.550° C., that reported by Elsdon and Stubbs is 0.544° C. Aschaffenburg and Veinoglou (1), MacDonald (8) and Stubbs and Elsdon (6) all consider that −0.530° C. is the limiting freezing point of milk and that no sample with a freezing point depression smaller than 0.530° C. should be considered watered. As a matter of interest this value likewise was employed in the calculations to be presented.

The data obtained are shown in fig. 3. They are presented as the algebraic means of the differences between the percentage water calculated and that actually added. Each curve is a mean of 16 runs; the milks were identical with those used for obtaining the data for figures 1 and 2.

These data support the contention of Elsdon and Stubbs that the A.O.A.C. procedure is in error, that the error increases as the added water increases and that their formula, which takes into account the change in water content of the milk, virtually eliminates the errors introduced by the A.O.A.C. formula. It is necessary to determine total solids in conjunction with the Elsdon and Stubbs procedure.

The values obtained with the Elsdon and Stubbs formula (VI) approximated

the actual quantities closely when the average freezing point depression 0.550° was employed; the values were approximately 1.0 per cent low when the value 0.544° C. was employed.

The accuracy of the Elsdon and Stubbs formula when the average freezing point depression, 0.550° C., was employed is indicated by the data in table 3. These data indicate that the Elsdon and Stubbs formula used in conjunction

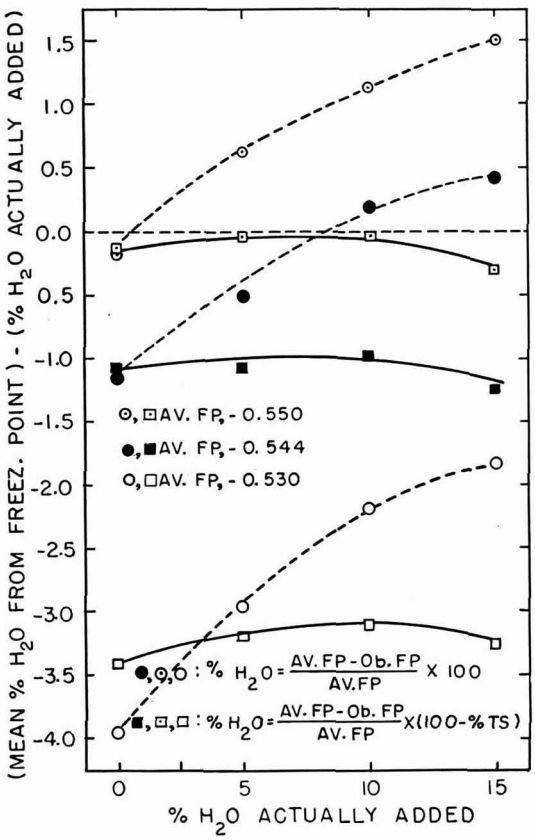

FIG. 3. Algebraic means of errors encountered when the percentage water added to milk was calculated by the formulae of the A.O.A.C. and of Elsdon and Stubbs; two possible average and one limiting freezing points were employed.

with the average freezing point depression of 0.550° C. would be expected to detect 3.0 per cent added water 99 times in 100 and 2.0 per cent 95 times in 100 if the sample variances are equal to the population variances. The modified lactometric procedure by comparison would be expected to detect 5.0 per cent added water 95 times in 100 with the local milk supply, if there was the same agreement among sample and population variances.

The regression equation (calculated from the data from which table 3 was compiled) relating the percentage added water, calculated by the equation employed in table 3, and the amount of water actually added is:

% H_2O, freez. pt. = 0.9903 (% H_2O added) − 0.053.

The standard error of estimate is 0.995 and the correlation coefficient is 0.9862.

A similar equation correlating the modified lactometer method with the cryoscopic procedure was calculated:

% H_2O, lactom. = 1.019 (% H_2O, freez. pt.) + 0.0173.

The standard error of estimate is 1.826; the correlation coefficient is 0.9535.

TABLE 3

Means and fiducial limits[a] *of the percentage added water, estimated by the Elsdon and Stubbs formula,*[b] *at different levels of watering*[c]

Prob. level used	Normal milk	Normal milk +5% H_2O	Normal milk +10% H_2O	Normal milk +15% H_2O
95%	−0.133 ± 1.837	4.932 ± 1.992	9.966 ± 2.016	14.713 ± 2.291
99%	−0.133 ± 2.540	4.932 ± 2.755	9.966 ± 2.788	14.713 ± 3.168

[a] For a single determination, assuming that the sample variances are equal to the population variances.

[b] % added $H_2O = \frac{0.550 - T_1}{0.550} \cdot (100 - T.S.)$.

[c] H_2O added as % by weight.

SUMMARY

Lactometric and cryoscopic methods were studied relative to the adaptability and accuracy (of the lactometric procedures) for the determination of water added to milk.

The limiting formula: % added water $= \frac{SS_1 - SS_2}{SS_1} \cdot 100$ was used in conjunction with the lactometric methods. SS_1 was calculated from the Babcock fat test by the Jacobson regression equation: $SS_1 = 0.4F + 7.07$; SS_2 was evaluated by the lactometric method for the sample under observation.

The seven common lactometric procedures employed yielded results that were low; the variations from the percentages of water actually added increased in non-linear fashion as the percentage of water added increased.

The modified Herrington formula, used with milk samples which were heated to 45° C., cooled to 30° C. and read at that temperature, yielded results that were slightly high for normal milk and decreased linearly as the percentage added water increased. This variation resulted from the fact that the fat percentage from which SS_1 was calculated was low in the watered milk.

As a result, the limiting formula was modified to yield the following formula:

$$\% \text{ added } H_2O = 110.89 - 111.11 \cdot \frac{SS_2}{SS_1}$$

which gave fairly satisfactory results when used in conjunction with the modified Herrington formula for the determination of SS_2.

With this procedure, 32 of 33 watered (4 to 15%) samples were diagnosed as

watered, the 33rd as suspected as watered, five of nine skimmed samples were designated skimmed or suspected as skimmed, two as normal and two as suspected as watered, and of six normal samples, four were designated as normal, one as suspected as skimmed and one as suspected as watered.

It is considered, therefore, that the procedure would serve well, for the milk supply studied, as a sorting method, that samples designated as having 5 per cent or more added water, would be watered 95 in 100 times and that the results are sufficiently promising to warrant other investigators checking the method in other milk sheds.

The Elsdon and Stubbs formula, for calculating added water from the freezing point depression of the milk, was found superior to that of the A.O.A.C. when the value 0.550° C. was employed as the average freezing point depression of milk. The data obtained indicate that the method will detect 3 per cent added water at the 99 per cent probability level and 2 per cent at the 95 per cent probability level.

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DETERMINATION OF TOTAL SOLIDS IN NORMAL AND WATERED MILKS BY LACTOMETRIC METHODS[1, 2]

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Behrend and Morgen (5) were probably the first to recognize the relationship existing between the total solids and specific gravity of milk. Fleischmann (7) appears to be the first individual to present a formula relating fat percentage, specific gravity and total solids that was sufficiently accurate to be generally accepted. Babcock (2, 3) and Richmond (14, p. 121) advanced formulae most commonly employed in the United States and England, respectively, during recent years. Toyonaga is credited by Fleischmann and Wiegner (8) as the individual who first recognized the importance of the physical condition of the fat in the lactometric determination of total solids. Studies relative to the physical condition of the fat were presented, in addition, by Bakke and Honegger (4), Lampert (11) and Sharp and Hart (16).

None of these workers, however, presented data with regard to the accuracy of the lactometric procedure for the determination of total solids in watered milks; in this connection, only one reference has been discovered. Desai and Patel (6) reported that the results obtained by Richmond's formula generally were higher than those determined by gravimetric methods. The variation between the methods was greater for watered than for normal milks.

The ultimate goal of the project, of which this study is a part, was to determine whether or not the lactometer could be employed to estimate the amounts of water added to milk (17). Since the adulteration portion of the project depended on the accuracy with which the total solids could be determined in normal and watered milks, this study was undertaken.

EXPERIMENTAL PROCEDURE

Sixteen runs were made comparing the lactometric and Mojonnier (13) methods for total solids in milk. One run was made each week, during the interval October 1, 1949 to March 15, 1950. Ten of the 16 samples were obtained from the Iowa State College herd; there were two samples each from Holstein, Jersey, Brown Swiss, Guernsey and Ayrshire cattle. The remaining six samples were selected from patrons who delivered milk to the College Dairy; these milks were known to be unadulterated.

Approximately 12 qt. of milk were needed for each run. This large sample

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[2] These data are part of a thesis submitted by the senior author for the degree Master of Science in Dairy Industry.

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was divided into four approximately equal portions. Portion 1 was unadulterated; to portions 2, 3 and 4, water was added to the extent of 5, 10 and 15 per cent by weight, respectively. Each portion so prepared was divided among three quart milk bottles and was held at ca. 40° F. over night.

Babcock fat tests (1, p. 312–316) were determined for each portion on the day the samples were prepared. Lactometer readings and Mojonnier, gravimetric total solids (13, p. 122–126) were determined the following day.

Three lactometric procedures were employed:

(a) The milk samples were removed from the refrigerator (ca. 40° F.), after overnight storage, thoroughly mixed and warmed (in stoppered lactometer cylinders) to 15.5° C. in a glass water bath with a merc to merc regulator. When at the desired temperature, the samples were stirred several times with a stirring rod, at the bottom of which a perforated rubber disk was attached. The agitation was carefully done, to prevent incorporation of air.

The lactometer was tempered in a cylinder of distilled water placed in the bath during the time the samples were warmed. When the samples were at the desired temperature, the lactometer was removed from the cylinder, quickly dried and placed immediately into the first well-mixed sample. The reading was taken as soon as the lactometer was at rest. The lactometer was removed from the first sample, drained (ca. 1 min.) and placed in the second well-mixed sample. This process was repeated until the series (4 or 8 samples) was read.

(b) The milk samples were removed from the refrigerator, mixed thoroughly and warmed to 45° C., in the milk bottles in which they had been stored. They were held at this temperature for 2 min., were mixed, poured into lactometer cylinders, stoppered and tempered to 15.5° C. (ca. 30–40 min.) as was done in procedure *a*. The remaining portion of the procedure was as described above.

(c) This procedure was identical with *b*, except that the samples were read in the water bath at 30° C. instead of at 15.5° C.

The lactometers employed at 15.5° C. were made by the Kimble Glass Co. These had a lactometer scale from 20 to 37° Q, calibrated at 60° F./60° F. in 1° intervals. Those employed at 30° C. were manufactured by Rascher and Betzold. The scale range was 13° to 28° Q, calibrated at 60° F./60° F. in 0.1° intervals. These lactometers should be calibrated from 17° to 32° Q. for greater utility. In all procedures the lactometers were read at the top of the meniscus to the nearest 0.1° Q.

Kimble, no. 20065 lactometer cylinders, 480-ml. capacity, total height 300 mm., i.d. 46 mm., were employed for all lactometer readings.

The formulae that were employed to calculate the percentages of total solids from the lactometer readings were:

With procedures *a* and *b*, those of Babcock (I and II) and of Richmond (III):

$$\text{I. T. S.} = 1.1842\ F + 0.2631\ L, \quad (2)$$
$$\text{II. T. S.} = 1.2\ F + 0.25\ L, \quad (3)$$
$$\text{III. T. S.} = 1.2\ F + 0.25\ L + 0.14, \quad (14, \text{p. } 121)$$

With procedure *c*, the calculations were made in accordance with the formulae

of Sharp and Hart (IV), Herrington (V), Rueda (VI) and Livak (VII):

$$\text{IV. T. S.} = 1.2537\ F + 0.268\ L/S, \quad (16)$$

$$\text{V. T. S.} = 1.2537\ F + \frac{268\ (L + 3.0)}{L + 1000}, \quad (9)$$

$$\text{VI. T. S.} = 1.2537\ F + 0.268\ \frac{(L + 3.2)}{S}, \quad (15)$$

$$\text{VII. T. S.} = 1.2537\ F + 0.268\ \frac{(L + 3.5)}{S}, \quad (12)$$

In equations IV to VII, L represents the lactometer reading at 30° C. In equation IV, S represents the specific gravity at 30° C./30° C., while in equations VI and VII, it represents the specific gravity at 30° C./15.5° C. In order to obtain the specific gravities at 30° C./30° C. for equation IV, the lactometers were calibrated at 30° C. in sucrose solutions. The lactometer readings then were plotted against the specific gravities of the sucrose solutions at 30° C./30° C. The lactometers were calibrated at three points, the data were plotted and it was assumed that the change in specific gravity scale was linear between each two calibration points. The sucrose solutions employed and the specific gravities found are presented in table 1. The lactometer readings were corrected for each determination made from the curves plotted.

TABLE 1

Specific gravities of four sucrose solutions at different temperatures

Soln no.	Sucrose[a]	Specific gravity[b] at		
		15.5° C./15.5° C.[c]	30° C./15.5° C.[c]	30° C./30° C.[d]
	(*g./l.*)			
1	61.31	.....	1.0202	1.0237
2	68.00	1.0266	1.0228	1.0263
3	75.00	1.0294	1.0254	1.0289
4	82.40	1.0318	.....	.....

[a] Phanstiehl, c.p. sucrose, specific rotation + 66.5°.
[b] Each value the average of 4 detn.
[c] Detn'd. pycnometrically.
[d] Calculated by multiplying values at 30° C./15.5° C. by the fraction sp. gr. H_2O, 15.5° C./sp. gr. H_2O, 30° C. Densities of H_2O from International Critical Tables (10).

A temperature of 15.5° C. was maintained in the water bath during warm weather by circulating water at *ca.* 5° C. (from an insulated bath (*ca.* 20 gal.) cooled with large brine pads) through the cooling coil.

RESULTS

The results obtained are shown in fig. 1. They are presented as the algebraic means of the differences between the total solids values as determined by the lactometric methods and those determined by the Mojonnier method (13).

These data indicate that the results by the Richmond formula (III) yielded the closest approximation for total solids in normal milk of any of the formulae employed, if the sample was heated to 45° C. and then cooled to 15.5° C.

The forms of the curves for all procedures involving the Babcock (I, II) and Richmond (III) formulae are similar. The error in the percentage total solids

increases rapidly as the percentage water added to the milk increases. These data agree with the statement of Desai and Patel (6) viz., that the Richmond (III) formula yields results that are high for normal milk and that the difference between the total solids by this formula and by a gravimetric method increases as the amount of water added to milk increases.

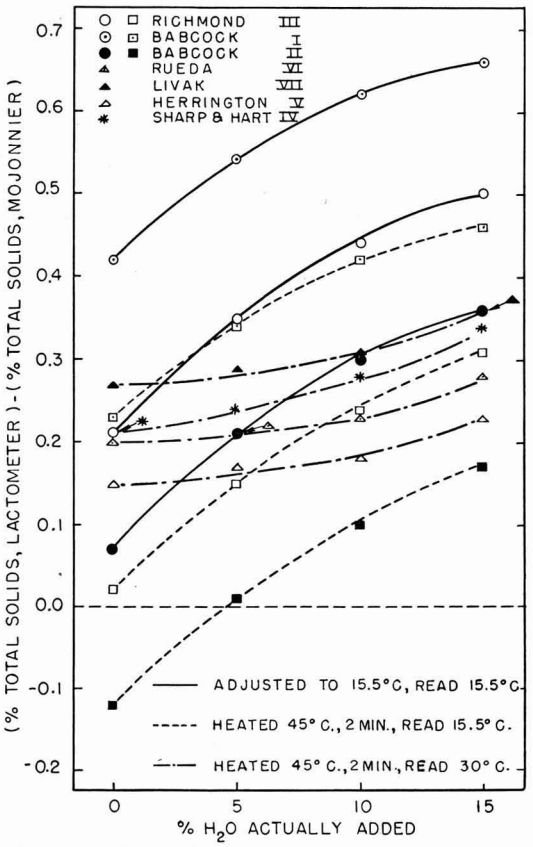

FIG 1. Algebraic means of errors in estimating the percentages of total solids in normal and watered milk by lactometric procedures.

When the milks are heated to 45° C., cooled to 15.5° C. and read at the latter temperature, the curves for formulae I, II and III are lowered considerably with respect to the ordinate, but the form of the curves remains essentially the same. It would appear, therefore, that the errors encountered result largely from the fact that the equations do not apply to watered milks rather than to the physical condition of the fat.

The values for total solids, determined when the milk was heated to 45° C., cooled to 30° C. and read at that temperature, were again high. The increase in variation from the Mojonnier total solids values, however, was slight as the water

added to the samples was increased; the Sharp and Hart formula (IV) showed the greatest variation with increase in added water. The Herrington formula checked the Mojonnier values most closely among formulae IV to VII, inclusive. The average total solids value obtained by the Herrington formula varied from that by the Mojonnier method by a value that was essentially 0.15 per cent for normal milk and milks containing 5 and 10 per cent added water. It was considered, therefore, that if 0.15 were subtracted from the values obtained by the Herrington formula, when the measurement was made with samples that were heated to 45° C. and read at 30° C., the total solids values estimated would closely check those of the Mojonnier gravimetric test. Such a correction would yield the following modified Herrington formula (VIII):

$$\text{VIII.}\quad \text{T. S.} = 1.2537\ F + \frac{268\ (L + 3.0)}{L + 1000} - 0.15$$

The total solids values were recalculated for all samples that had been run by formula VIII. The data are presented in table 2. These data indicate that, for

TABLE 2

Algebraic means and ranges of differences. (Total solids, formula VIII–total solids, Mojonnier method)

Normal milks[a]		Normal milks[a] +5% H_2O		Normal milks[a] +10% H_2O		Normal milks[a] +15% H_2O	
Mean	Range	Mean	Range	Mean	Range	Mean	Range
−0.013	0.11 to −0.20	+0.021	0.15 to −0.17	+0.029	0.17 to −0.19	+0.078	0.33 to −0.11

[a] A total of 16 runs is represented by these data.

the samples tested, the modified Herrington formula (VIII) yielded satisfactory results for normal milks and for milks containing up to 15 per cent added water. The regression equation relating the total solids by formula VIII and the Mojonnier method, for all milk samples represented in table 2 is:

% T. S. (formula VIII) = 0.9835 (% T. S. Mojon) + 0.225.

The standard deviation of the deviations from the regression is 0.102. When the data for normal and watered milks were pooled, the correlation coefficient between the two methods is 0.9958.

SUMMARY

Several lactometric procedures were checked as regards their accuracy for the determination of total solids in normal and watered milk.

Babcock formula II yielded satisfactory results for normal milk, if the sample was warmed to 15.5° C. and read; Richmond's formula (III) yielded the best results among the formulae checked if the milks were heated to 45° C., held 2 min., cooled to 15.5° C. and read.

The Babcock and Richmond formulae yielded high values for watered milk by either of the methods indicated in the preceding paragraph, with the exception of Babcock formula II, which checked well at the 5 per cent water addition.

The deviations of the results by these formulae from these by the Mojonnier method increased sharply as the percentage water added to the milk increased.

When the samples were heated to 45° C., held 2 min., cooled to 30° C. and read at that temperature, the results by the Herrington formula check the Mojonnier results more closely than those calculated in accordance with Sharp and Hart, Rueda or Livak. Although the differences between the total values by the lactometric and Mojonnier methods increased as the percentage added water increased, this increase was fairly small.

When a constant, 0.15, was subtracted from the results calculated by the Herrington formula, the values obtained checked those by the Mojonnier method closely. This modified formula (VIII) was satisfactory for the determination of total solids in normal milks or in milks containing up to 15 per cent added water, for the milk received at the College Dairy.

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THE USE OF THE BRABENDER SEMI-AUTOMATIC MOISTURE TESTER FOR THE DETERMINATION OF TOTAL SOLIDS IN MILK[1, 2]

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It was necessary to check techniques for the determination of total solids in milk, in connection with another problem. It was considered advisable, therefore, to study total solids determination in milk by the Brabender tester, at the same time.

Kosikowsky *et al.* (3) have checked the Brabender instrument for determining moisture in cheese; no report has been encountered relative to its use for milk.

EXPERIMENTAL PROCEDURE

Three methods for the determination of total solids were employed, *viz.*, the A.O.A.C. (1, p. 30), Brabender (2) and Mojonnier (4, p. 122–126).

A concentric ring type, constant temperature bath (Chicago Surgical and Electrical Co., type 139) and a Beockel drying oven (no. 1078) were employed for the A.O.A.C. method.

The Brabender preliminary runs were conducted with 2-, 5-, and 10-g. samples; the final runs with 10-g. samples. The 2-g. samples were weighed with an analytical balance; the 5- and 10-g. samples with a Torsion butter moisture scale. Variation in samples size was compensated for as indicated in the directions issued with the tester (2).

The dishes employed in the Brabender procedure were cleaned by soaking for 0.5 to 1.0 hr. in a solution of Nacconol or Dreft and were brushed free of deposit with a stiff brush. The dishes were dried in the Brabender tester. The tester was adjusted with these dishes before the samples were weighed into them. The dish weights were checked periodically and were found to remain constant with the cleaning procedure employed.

Homogenized milk from the College Dairy was employed for all tests made. In the comparison involving sample weight, drying temperature and drying time (fig. 1), one large lot of homogenized milk was used for all 12 runs. The large sample was thoroughly mixed and was divided into 12 portions, one for each of the runs made. There were four runs for each temperature, *viz.* 100, 105 and 110° C.; one portion of milk was used for a single run at a definite temperature. Triplicate determinations were made for each sample weight in each run.

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[2] These data are part of a thesis submitted by the senior author for the degree Master of Science in Dairy Industry.

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RESULTS

Determination of optimum conditions for the Brabender method. There was no guide as to the time, temperature or sample weight that should be employed with this procedure. Experience with condensed whey indicated severe browning and lack of definite equilibrium with this product when the Brabender apparatus was employed. For this reason an attempt was made to keep the temperature as low as possible.

Temperatures of 100, 105 and 110° C. and sample sized of 2, 5 and 10 g. were employed.

Four runs were made at each temperature. The averages of the four runs are presented graphically in fig. 1. On the basis of these data, a sample size of

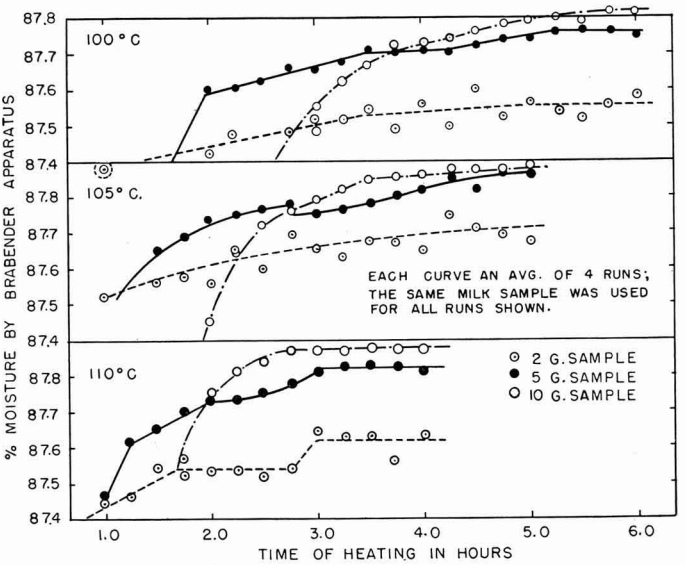

FIG. 1. Percentages of moisture obtained by the Brabender Tester when temperature, sample weights and time of heating were varied.

10 g. and a drying time of 3 hr. were selected as satisfactory for comparing the Brabender procedure with the A.O.A.C. and the Mojonnier procedures. These data indicate that the moisture values obtained at any temperature are a function of the sample weight; the smaller the sample, the lower the moisture content indicated for the sample.

The variations among replicate determinations of the same sample of milk were greater the smaller the sample weight. This is logical, since the moisture percentages could be read to 0.05, 0.10 and 0.25 per cent, respectively, for the 10-, 5- and 2-g. samples.

At the lower temperatures, equilibrium conditions were not established during 5 to 6 hr. Temperatures higher than 110° C. were not studied, since it was considered that with a fluid medium, spattering might result.

The 5- and 10-g. samples became a pronounced brownish-red during the determinations, suggesting a lactose-protein reaction, though to a much lesser degree than is exhibited by condensed whey or whey-containing products.

TABLE 1

Total solids of milk by the Brabender, A.O.A.C. and Mojonnier methods

Milk sample	Brabender apparatus 3 hr. 110° C. (2)		A.O.A.C. method (1)		Mojonnier method (4)	
	Av. of triplicates		Av. of triplicates		Av. of triplicates	
	A	B	A	B	A	B
1949						
July 5	12.18	12.20	12.64	12.58	12.53	12.50
" 8	12.20	12.17	12.31	12.35	12.57	12.53
" 10	12.00	12.00	12.32	12.32	12.31	12.26
" 12	12.00	12.00	12.07	12.05	12.13	12.19
" 15	12.20	12.18	12.37	12.46	12.46	12.41
Aug. 11	11.80	11.88	12.14	12.17	12.19	12.23
Sept. 2	12.13	12.12	12.27	12.29	12.27	12.28
Oct. 4	12.23	12.25	12.35	12.36	12.37	12.40
Nov. 11	12.20	12.23	12.39	12.38	12.27	12.29
Dec. 22	12.40	12.40	12.60	12.59	12.41	12.40
1950						
Jan. 12	12.15	12.13	12.21	12.20	12.15	12.17
Feb. 9	12.42	12.42	12.49	12.52	12.47	12.47
Means	12.159	12.165	12.347	12.356	12.344	12.344

Comparison of the A.O.A.C., Brabender and Mojonnier methods for total solids in milk. Homogenized milk samples from the College Dairy were thoroughly mixed, and were divided into three parts, one for each test. Each part was divided into two samples, A and B. Triplicate determinations were made with samples A and B. Five runs were made during July, 1949, and one run was made each month thereafter to and including February, 1950. The data are summarized in table 1.

TABLE 2

Analysis of variance of estimated total solids percentages presented in table 1

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total sum of squares	215	6.7397	
Dates	11	3.9327	0.3575
Methods	2	1.6358	0.8179
A.O.A.C. vs. Brab.	1	1.2713	1.2713
A.O.A.C. vs. Mojon.	1	0.0016	0.0016
Dates × methods	22	0.9194	0.0418
Aliquot A vs. Aliquot B within methods × dates	36	0.0538	0.0015
Brabender	12	0.0159	0.0013
A.O.A.C.	12	0.0215	0.0018
Mojonnier	12	0.0165	0.0014
Determinations	144	0.1980	0.0014
Brabender	48	0.0330	0.000694
A.O.A.C.	48	0.0908	0.001892
Mojonnier	48	0.0739	0.001540

An analysis of variance (table 2), of the data presented in table 1, indicates that the least variation among replicate determinations of the same sample of

milk is obtained with the Brabender and the greatest variation by the A.O.A.C. method. It is estimated with 99 per cent confidence that the ranges indicated by a single determination ($\pm$ 0.071 per cent for the Brabender, $\pm$ 0.111 per cent for the A.O.A.C. method and $\pm$ 0.105 per cent for the Mojonnier method) will encompass the true population means for these methods.

The mean by the Brabender procedure is significantly lower than that obtained by the A.O.A.C. procedure. This may result from the greater decomposition (indicated by sample color) that appeared to occur in the samples run by the Brabender procedure.

No statistically significant difference was encountered between the A.O.A.C. and Mojonnier means, which is in agreement with data that have been presented by other workers.

SUMMARY

A check of the use of the Brabender tester for determination of total solids in milk indicated that the optima among the conditions studied were: A 10-g. sample weight, 110° C. drying temperature, and 3.0 hr. drying time.

The values for total solids obtained by this method were significantly lower than those by the A.O.A.C. procedure (mean A.O.A.C. – mean Brabender = 0.19%).

The variations among the replicate determinations of the same sample of milk were least by the Brabender, greatest by the A.O.A.C. and intermediate by the Mojonnier method.

There was no significant difference between the mean determinations by the Mojonnier and A.O.A.C. procedures.

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THE ASSOCIATIVE ACTION BETWEEN CERTAIN YEASTS AND *BACTERIUM LINENS*

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The presence of budding and salt-tolerant yeasts in large numbers on the surface of limburger cheese during the early stages of ripening has been suggested by Filipović (3) and Kelly and Marquardt (7) as essential in the preparation of the cheese surface for the subsequent growth of *Bacterium linens,* the proteolytic activity of which completes the ripening process. Since the acidity of 1-day-old limburger cheese is approximately pH 5.0, and *B. linens* does not grow in media more acid than pH 5.85, Kelly and Marquardt (7) have suggested that the yeasts act on the proteins and lactates to decrease the acidity and thus facilitate the growth of *B. linens.* Recently, Iya and Frazier (4) isolated a yeast from the surface of brick cheese, also a surface-ripened type, which caused a decrease in acidity of several acid media.

Iya and Frazier (4) also have reported that, in addition to decreasing the acidity of brick cheese, the yeast may promote the growth of *B. linens* by supplying needed growth factors. Evidence in support of this possibility is presented by Burkholder *et al.* (2) and Meyer (10) who have found that certain strains of *B. linens* require pantothenic acid for growth.

Thus, it appears that the yeasts contribute indirectly to ripening in at least two ways: (a) by decreasing the acidity of the curd and (b) by synthesizing growth factors. Each of these activities may be necessary to initiate the growth of *B. linens.* However, the evidence available is indirect.

It is the purpose of this paper to report the results of experiments designed to test directly the hypothesis that the yeasts of limburger cheese contribute to the growth of *B. linens* by decreasing the acidity of the curd and by simultaneously secreting growth factors which are necessary for the growth of *B. linens.* To determine if these factors are of general importance, several yeasts and several *B. linens* cultures have been studied. In addition, vitamin secretion during growth of the yeasts has been measured in order to identify the yeast-secreted growth factors which promote the growth of *B. linens.*

In this study it was found that virtually all of the yeasts decreased the acidity of various media to a point which would allow the growth of *B. linens.* In addition, it was found that the yeasts supply factors necessary for growth of all of the vitamin-dependent *B. linens* strains. Appropriate tests, including microbiological assays, showed that the same yeasts were able to synthesize pantothenic acid, niacin, riboflavin and, in some cases, biotin. The evidence indicates that pantothenic acid is the growth factor which is involved in the associative action.

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METHODS AND MATERIALS

Organisms. Twenty-nine yeasts were isolated from commercial limburger cheese and from unripened limburger cheese curds essentially by the method of White and Hood (11). Twenty-seven of the yeasts reproduced by budding, were salt-tolerant, and were otherwise similar to those described by Kelly (6). Two isolates were fission yeasts which, in addition to being salt-tolerant, also hydrolyzed butterfat and rapidly liquified gelatin. For the studies of vitamin synthesis reported below, inocula of the 29 yeasts were grown in a tryptone, beef-extract, glucose-medium for 48 hr. at 30° C. The cells were harvested by centrifugation, washed twice with sterile distilled water and diluted to an optical density of 0.1. Density readings were made in the Lumetron colorimeter with a no. 370 filter. One drop of this suspension was used as an inoculum.

Twenty-five cultures of *B. linens* were isolated by Meyer (10) from limburger cheese by the method of Albert *et al.* (1). Fourteen of these strains were incapable of growth in a vitamin-free medium. In addition, two strains, nos. 9174 and 9175, were obtained from the American Type Culture Collection. The

TABLE 1

Vitamin-free, semi-synthetic agar for growth of B. linens

Basal medium		Additions[c]	
	(*g./l.*)		(γ/*l.*)
Glucose	20	Riboflavin	100
Casein hydrolysate, enzymatic	5	Thiamin HCl	100
L-tryptophane	0.1	Niacin	100
L-cystine	0.2	Pantothenic acid	100
Adenine sulfate	0.010	Biotin	5
Guanine HCl	0.010	Pyridoxine	100
Uracil	0.010	Inositol	10,000
Agar	15	Choline	5
Salts A[a]	5 ml.	Folic acid	5
Salts B[b]	5 ml.	p-aminobenzoic acid	10

[a] Salts A: 25 g. of K_2HPO_4 and 25 g. of KH_2PO_4 in 250 ml. of H_2O.
[b] Salts B: 10 g. of $MgSO_4 \cdot 7H_2O$, 0.5 g. of NaCl, 0.5 g. of $FeSO_4 \cdot 7H_2O$, and 0.5 g. of $MnSO_4 \cdot 4H_2O$ in 250 ml. of H_2O.
[c] As indicated in text.

inocula were prepared by growing the cultures at 20° C. for 72 hr. in a medium composed of 1 per cent each of yeast extract and tryptone and 0.5 per cent each of dipotassium phosphate and glucose. The cells were centrifuged, washed and resuspended to an optical density of 0.1 as described above for the yeasts. One ml. of this suspension was used to seed the agar for each plate.

Media. For studies of the interaction between the yeasts and *B. linens,* a vitamin-free, semi-synthetic agar[1] was employed (table 1). This medium afforded good yeast growth and permitted the addition of vitamins as desired. The acidity of the medium was adjusted to either pH 6.5 or to pH 5.0 as later indicated.

For the production of pantothenic acid, niacin, riboflavin and biotin, the

[1] Bacto-agar, known to contain traces of vitamins, was employed. Since growth of *B. linens* was not obtained without added vitamins, the contamination is not considered to be significant.

yeasts were grown in several complex media (5), each type of which was deficient in the vitamin under test (table 2). The media were adjusted to pH 6.8, dispensed in 50-ml. amounts in 125-ml. flasks and sterilized by autoclaving. Microbiological assays for the presence of vitamins were carried out on the media following growth of the yeasts. The cells were removed by filtration through a Selas filter of #03 porosity. The filtrates were adjusted to pH 6.8 and appropriate aliquots added to microbiological assay media prior to autoclaving.

Microbiological assays for the presence of pantothenic acid, niacin, riboflavin and biotin were performed in the media shown in table 2. Thus, the media used

TABLE 2

Media for vitamin synthesis by yeasts

Component	Medium			
	A[c]	B	C	D
		(*mg./l.*)		
Glucose	20,000	20,000	20,000	10,000
Casein (acid-hydrolyzed, vitamin free)	2,500	2,500	1,000	–
Peptone (alkali treated)	–	–	5,000	5,000
Sodium acetate, anhydrous	20,000	20,000	12,000	6,000
L-tryptophane	100	100	–	–
L-cystine	200	200	20	200
Adenine sulfate	5	10	–	–
Guanine HCl	5	10	–	–
Uracil	5	10	–	–
Xanthine	5	–	–	–
Yeast extract (alkali treated)	–	–	1,000	2,000
Calcium pantothenate	1	0.1	–	0.5
Thiamin HCl	1	–	0.25	–
Niacin	1	–	0.25	–
p-aminobenzoic acid	0.1	0.1	0.1	–
Biotin	–	0.001	–	–
Riboflavin	–	0.2	0.1	–
Pyridoxal HCl	1	–	0.2	–
Pyridoxine	–	0.1	–	–
Salts A[a]	5 ml.	5 ml.	5 ml.	5 ml.
Salts B[b]	5 ml.	5 ml.	–	5 ml.

[a, b] See table 1.

[c] For assay of: A = biotin, B = niacin, C = pantothenic acid, D = riboflavin.

in these assays were of the same composition as the aliquots to be assayed. Pantothenic acid, niacin and biotin were assayed with *Lactobacillus arabinosus* (ATCC *8014*) and riboflavin was assayed with *Lactobacillus casei* (ATCC *7469*). In all assays the methods outlined by Johnson (5) were employed.

RESULTS

The first experiment was designed to test the suggestion (7) that the yeasts perform an essential function by decreasing the acidity of the curd. For this purpose, a synthetic medium (table 1) containing a vitamin mixture or pantothenic acid (table 2) adjusted either to pH 5.0 or 6.5 was employed. Following, this, the agar was heavily seeded with *B. linens* and plated. After solidification of the agar, the plates were spot inoculated with various yeasts and incubated 96 hr. at 20° C. The results of a typical experiment using *B. linens,* strain 456,

and yeast "G" are shown in fig. 1. As can be seen from the photograph, growth of the seeded organism did not occur in the media adjusted to pH 5.0 (plate 1). However, when a similar plate was spot inoculated with a yeast, a marked zone of growth occurred around the yeast colony (plate 2). The situation is different at pH 6.5. At this pH, *B. linens* grew whether or not yeast was present (plates 3 and 4). As can be seen, the growth of *B. linens* was uniform on plates 3 and 4 thus indicating that the medium was satisfactory for growth of this organism. As the only difference between the two media was in the degree of acidity, it was

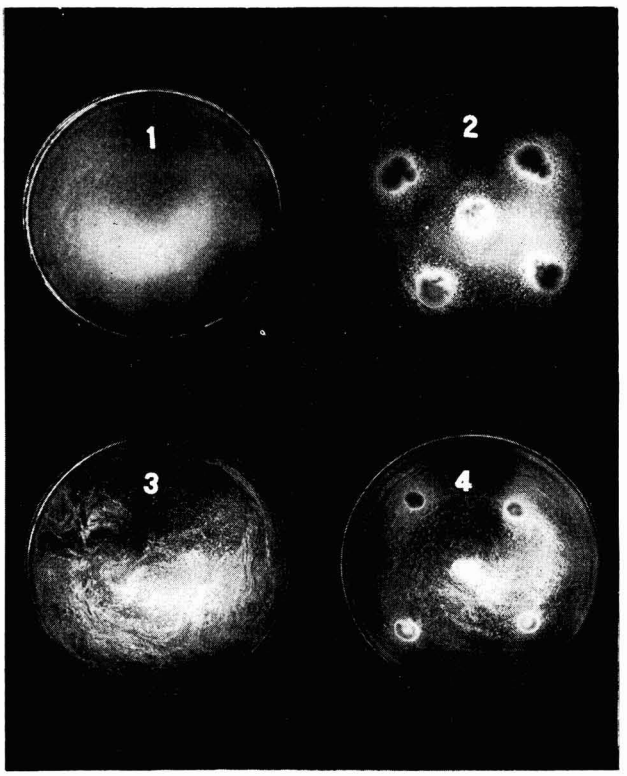

FIG. 1. The importance of acidity in the associative growth of yeasts and *B. linens*.

concluded, in confirmation of the observations of Kelly and Marquardt (7), that the yeast decreased the acidity of the medium. This conclusion was supported further by the observation that the yeast growing in a mineral-sodium lactate medium containing vitamins and in washed cottage cheese curds shifted the acidity from pH 5.0 to pH 6.2.

Although the curds of limburger cheese contain vitamins (2), these may not be present in sufficient concentration to afford rapid growth of vitamin-dependent organisms. To test the possibility that the yeasts promote the growth of *B. linens* by secreting vitamins, a vitamin-free medium adjusted to pH 6.5 was used. The medium, with and without added vitamins was seeded with *B. linens*

and plated as previously described. The result of a typical experiment is shown in fig. 2. Growth did not occur in the vitamin-free medium even though the pH

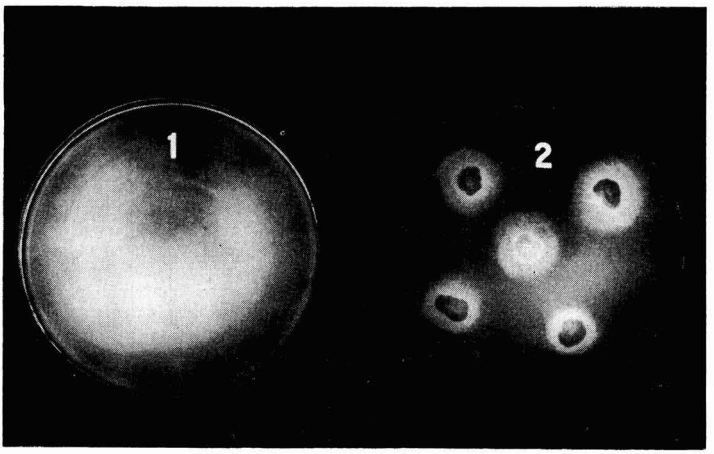

FIG. 2. The importance of pantothenic acid in the associative growth of yeast and *B. linens*.

was optimal for growth (plate 1). However, when the same medium was spot inoculated with a yeast, *B. linens* grew around the yeast colony (plate 2). In addition, growth of *B. linens* occurred throughout the plate when the agar was supplemented either with all vitamins or with pantothenic acid. Also, no increased growth of *B. linens* was observed on the vitamin-containing medium

TABLE 3

Growth of B. linens in various media

Initial pH	Additions	Growth of *Bacterium linens*	
		PA required[a]	No requirement[b]
5.0	none	–	–
5.0	all vit.	–	–
5.0	yeast	+	+
6.5	none	–	+
6.5	PA or all vit.	+	+
6.5	yeast	+	+

[a] *B. linens*, strains 456, 462, 470.
[b] *B. linens*, strains 451, 458, 459.

(plate 3, 4 of fig. 1). Thus, it appears that the yeast secretes pantothenic acid and that this vitamin is required by *B. linens* for growth.

The results of these studies with several cultures of *B. linens* demonstrating both the decrease in acidity and the vitamin secretion by the yeast, are summarized in table 3. Similar results have been obtained with the majority of the yeasts and all of the *B. linens* strains.

To test further the vitamin-producing capacity of the yeasts, the organisms were grown in media used for the microbiological assay of pantothenic acid, niacin, riboflavin and biotin (table 2). Following the yeast growth, the cells were removed as outlined in the method section and the growth media assayed micro-

biologically for the presence of vitamins. The results of this experiment, shown in table 4, indicate that all of the yeasts were capable of producing significant

TABLE 4

Synthesis of vitamins by yeasts

Yeast culture	Vitamins present ($m\mu g/ml.$)			
	Pantothenate	Niacin	Riboflavin	Biotin
A	12	20	41	0
B	1	10	19	0
C	15	15	49	0
D	15	45	200	0
E	8	15	52	0
F	9	20	53	0
G	15	19	49	0
H	21	19	47	0
I	20	19	43	0
J	27	10	6	0
K	1	20	32	0
L	26	181	47	0
M	3	18	27	0
N	170	104	33	3
O	35	20	55	0
P	49	17	18	2
Q	15	43	16	0
R	15	13	20	0
S	14	40	12	0
T	13	33	25	0
U	44	36	47	0
V	54	40	53	0
W	10	20	50	0
X	190	104	32	4
Y	10	60	22	0
Z	14	16	20	0
AA	2	57	5	0
AB	5	17	16	0
AC	10	40	13	0

amounts of pantothenic acid (1 to 190 mμg per ml.), niacin (10 to 180 mμg per ml.) and riboflavin (5 to 200 mμg per ml.). Three yeasts produced biotin (2.0 to 4.0 mμg per ml.). The uninoculated media treated in the same manner were free of the vitamin under test. Since the vitamin-requiring *B. linens* strains which have been tested require pantothenic acid (2, 10), the production of this vitamin appears to be the most important.

DISCUSSION

In view of the fact that the growth of *B. linens* on limburger cheese is dependent upon the curd being a satisfactory culture medium for this organism, it is not surprising to find that growth also is dependent upon, or always associated with, the presence of other organisms. The fact that yeasts are always present where *B. linens* is growing has led to the speculation that a symbiotic relationship exists between these organisms. However, the observations of Kelly and Marquardt (7), Iya and Frazier (4) and others (2, 8, 9) have indicated that this relationship may involve the reduction of curd acidity and also the production of growth factors by the yeasts.

From the foregoing experiments the importance of vitamin synthesis by the

yeasts during cheese ripening as well as other functions performed by the yeasts cannot be determined. However, it is now possible in the light of these data to test the ripening process with only one of the two organisms present, either the yeast or *B. linens* and to determine the additional function of each organism.

SUMMARY

The data reported herein support the hypothesis that the yeasts of limburger cheese contribute to the growth of *Bacterium linens* by decreasing the acidity of the curd and by simultaneously secreting required growth factors.

The presence of a yeast is required for growth of all *B. linens* strains in a vitamin-sufficient medium adjusted to pH 5.0 but not in the same medium adjusted to pH 6.5. A majority of the yeasts possess this growth-promoting ability.

The presence of a yeast is required for the growth of vitamin-dependent *B. linens* strains in a vitamin-free medium adjusted to pH 6.5 unless pantothenic acid is present. All of the yeasts possess growth-promoting ability under these conditions.

All of the yeasts secrete appreciable amounts of pantothenic acid, riboflavin and niacin.

ACKNOWLEDGMENTS

The authors wish to acknowledge with thanks the interest and suggestions of R. V. Hussong and the assistance of A. L. Scatterday in preparing the photographs.

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RELATION OF PRODUCTION RECORDS ON COWS TO EFFICIENT MANAGEMENT OF THE DAIRY FARM[1]

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Results of studies conducted at the University of Illinois and elsewhere show that, on the average, the annual yield of milk per cow tends to increase progressively with the length of the period during which a program of production testing in a Dairy Herd Improvement Association has been followed. Unpublished data obtained from Illinois D.H.I.A. records show that the cows in 318 herds completing 10 or more yr. of production testing in 1948 yielded an average production per cow per year of 57 lb. of butterfat more in 1948 than they did for the first year tested. Herds in Iowa averaged only 312 lb. of butterfat per cow during the first year of production testing while herds tested from 6 to 10 yr. averaged 360 lb. of butterfat per cow in the last recorded year of production testing (1). Increasing the average production per cow usually means more efficient production and higher returns from the dairy enterprise.

However, most dairy herds can not be considered independently from the farm on which that herd is maintained. The dairy enterprise normally is but a part of the over-all farming enterprise, even though a major portion of the farm income results from the sale of dairy products or of dairy cattle.

The purpose of this investigation was to determine whether or not dairymen who had their herds enrolled in a program of production testing in a Dairy Herd Improvement Association for 10 or more yr. obtained greater total farm operating efficiency than did those dairymen operating under similar circumstances who had not production tested their cattle.

INVESTIGATIONAL PROCEDURE

Financial, crop and milk production records kept by dairy farmers in cooperation with the Farm Bureau Farm Management Service[2] were used as the basic data for the study. An average of the yearly records for the years 1942, 1943 and 1944, in one series of data and the average of 1943, 1944 and 1945, for another series from farms in northern Illinois on which 45 per cent or more of the total farm income was received from the sale of dairy products and dairy cattle were used.

The measures of operating efficiency were (a) the rate of interest earned on the total farm investment and (b) the net farm earnings per acre. The first was calculated by dividing the net earnings of the farm after expenses had

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1 The data in this paper are from a thesis submitted to the Graduate College of the University of Illinois by the senior author in partial fulfillment of the requirements for the degree of Master of Science in Dairy Science, October, 1950.

2 The Farm Bureau Farm Management Service is an Extension Project carried on cooperatively between the University of Illinois, College of Agriculture and the Farm Bureaus.

been paid by the total amount of money which had been invested in the farm and farm business. It is the rate of interest which was earned on the money invested in the business. The second measure was obtained by dividing the net earnings per farm by the number of acres in the farm.

The first part of this investigation was concerned with development of a satisfactory method for selecting from the records available paired farms that were comparable in certain other factors having to do with farm operating efficiency, but on one of which 10 consecutive yr. of production testing of the dairy herd had been completed, and on the other no production testing program had been followed. The data from 65 different farms in the northeastern part of Illinois were available for the study. Nineteen different factors were considered as having a bearing on operating efficiency. Gross correlation coefficients between these individual variables in the records and the two different measures of operating efficiency were calculated by the product-moment method of analysis, as outlined by Mills (2).

The second part of the investigation dealt with the actual comparison of the series of paired farms as to their operating efficiency when measured by the rate of interest earned on the total farm investment and the net farm earnings per acre. From the developed method of pairing, a series of 21 pairs of farms was selected from the original data on the 65 farms. Each member of a pair was comparable in the important items relating to farm operating efficiency except that on one farm of each pair the cows had been production tested in the D.H.I.A. program during a period of at least 10 consecutive yr., while on the other farm of the pair the cows were not tested. A second series of paired farms was selected by the same means from average data available for the years 1943, 1944 and 1945, from farms in northwestern Illinois, a more general farming area. This latter comparison constitutes an independent test of the conclusions drawn from the comparison made between the first 21 pairs of farms. The statistical significance of the differences found was tested by the method of the analysis of variance.

RESULTS AND DISCUSSION

The 19 factors recorded and considered as having a bearing on farm operating efficiency are listed in table 1. Also, shown are the calculated gross correlation coefficients between each such factor and the interest earned on the total farm investment and the net earnings per acre. As table 1 shows, only five of these factors were significantly related to both measures of farm operating efficiency. These five factors were: (a) returns for $100 spent for feed for dairy cattle; (b) labor costs per cow; (c) average pounds of butterfat produced per cow per year; (d) crop returns per acre per year; and (e) the average number of cows in the milking herd. Considered jointly by the method of multiple correlation, these five factors were related to interest earned on the total farm investment in the order of $R = 0.75$, and to net earnings per acre in the order of $R = 0.78$. Thus, these five factors considered jointly in these data for the 65 dairy farms were responsible for 56.5 per cent of the variation in the first and

62.4 per cent of the variation in the second measure of farm operating efficiency used.

Though these five factors undoubtedly were the most important of the 19 factors contributing to the variability found in farm operating efficiency in these data, they are not necessarily the only factors influencing farm earnings. In fact, the variability in innate managerial ability of the farm operators themselves, certainly an important variable in these data, was assessed only as the sum total of farm operating efficiency, the final measure with which all other measured variables were correlated. Nevertheless, consideration of these five

TABLE 1

The relative influence of factors affecting the rate of interest earned on the investment and net earnings per acre as measured by gross correlation coefficients

	Rate earned on investment	Net earnings per acre
Returns for $100 feed fed to dairy cattle	0.51**	0.57**
Labor costs/cow	−0.37**	−0.32**
Level of production (butterfat/cow/year)	0.32**	0.30*
Crop returns/acre	0.32**	0.48**
Size of the herd	0.29*	0.30*
Feed fed/acre to productive livestock	0.23*	0.21
Number days on pasture	0.21	0.14
Per cent of income from sale of dairy cattle	0.20	0.09
Per cent of land in legumes	0.18	0.17
Pounds of milk produced per pound concentrates fed	0.21	0.14
Capital investment/acre	0.03	0.34**
Machinery costs/acre	−0.18	−0.18
Operating capital/acre	0.25	−0.04
Soil rating	0.04	−0.17
Investment in buildings/acre	0.19	0.04
Size of the farm	0.06	0.11
Per cent of land tillable	0.01	0.10
Investment in dairy cattle/cow	0.08	0.06
Labor costs/acre	0.03	0.03

** Significant at the 1% level.
* Significant at the 5% level.

important factors provided a basis for selecting the paired farms required by the logical structure of the comparative analysis to be made.

Since two of these factors, (a) returns for $100 spent for feed for dairy cattle, and (c) average pounds of butterfat produced per cow per year, are the classical reasons justifying a continuous program of production testing, they should not be considered as basic factors to be standardized in selection of the pairs of farms for comparisons of farm operating efficiency. However, there were no logical reasons or evidence to support a contention that any of the three other important factors concerned with operating efficiency, were inevitable consequences of a continuous program of production testing. Therefore, these three factors were chosen as the basic ones for selecting pairs of farms for the comparisons to be made. The pairing of one farm with another was accomplished by taking a farm, the herd of which had tested in D.H.I.A., and then finding a non-D.H.I.A. farm in the sample which had approximately the same size herd, about the same labor cost per cow and about the same

crop returns per acre. This process was continued until each of the D.H.I.A. herds had been paired with a similar non-D.H.I.A. herd. In all, 21 pairs of farms from the original 65 were selected for further study and statistical comparison.

Table 2 shows that the group of farms with herds on test in D.H.I.A. had a higher rate of interest earned, higher net earnings per acre, higher average butterfat production, higher returns above feed costs and higher feed cost per cow. Interest rate earned on the investment was 14.74 per cent for the D.H.I.A. herds while the non-D.H.I.A. group earned only 9.72 per cent. The farms on which the herds had been tested in D.H.I.A. had a return of $30.21 per acre,

TABLE 2

Comparison of herds tested 10 or more yr. in D.H.I.A. with similar non-D.H.I.A. herds in northeastern Illinois (1942–43–44)

	Herds tested 10 or more yr. in D.H.I.A.	Non-D.H.I.A. herds	F[a] value
Items used in pairing a D.H.I.A. herd with a non-D.H.I.A. herd			
Crop returns/acre	$ 33.13	$ 33.19	0.03
Labor costs/cow	$ 94.30	$ 93.30	0.02
Number of cows milked	28.3	26.8	0.28
Items showing a significant difference			
Rate earned on investment (%)	14.74	9.72	16.94**
Net earnings/acre	$ 30.21	$ 19.11	13.42**
Returns for $100 feed fed to dairy cattle	$ 178.00	$ 162.00	4.60*
Operator's earnings	$4,874.00	$3,039.00	6.25*
Average butterfat produced (*lb.*)	336.9	277.4	18.10**
Average milk produced (*lb.*)	9,320	7,872	13.11**
Returns above feed cost/cow	$ 326.00	$ 253.00	21.57**
Feed cost/cow	$ 183.00	$ 158.00	10.68**
Items not showing a significant difference			
Size of the farm (*acres*)	197	197	0.11
Machinery costs/ acre	$ 13.39	$ 14.74	0.08
Labor costs/acre	$ 22.74	$ 21.23	0.45
Building costs/acre	$ 3.85	$ 3.66	0.16
Returns from hogs	$1,604.00	$1,240.00	0.66
Per cent of land in legumes	28.1	26.0	0.88
No. of herds	21	21	

** Significant at the 1 per cent level.
* Significant at the 5 per cent level.
[a] Values obtained by analysis of variance.

while the non-D.H.I.A. group earned $19.11 per acre. Analysis of variance showed that the differences which were found between the two groups of farms for both measures of farm earnings were significant at the 1 per cent level of probability that variations of such magnitude were not due to chance alone. Therefore, it may be concluded from these data that dairy farms on which D.H.I.A. testing had been done over a period of 10 or more years were more efficiently operated than were the farms on which no production testing had been done. Just what part of this difference is due to continuous testing over a period of 10 years and what part is due to the fact that the dairyman who joins a D.H.I.A. may be more progressive is not known. However, it can be

assumed that production and feed records on each cow in the herd helps the most progressive man do a better job of managing the herd, which would result in more efficient production.

In table 3, 14 D.H.I.A. farms from northwestern Illinois are compared with 14 non-D.H.I.A. farms by the same method of pairing as was used for the 21 northeastern Illinois farms. This comparison constituted a different sample of farms in a more general farming area and an independent check on the validity of the methods of pairing and on the conclusions reached from the earlier comparison of 21 pairs of farms. The results show essentially the

TABLE 3

Comparison of herds tested 10 or more yr. in D.H.I.A. with similar non-D.H.I.A. herds in northwestern Illinois (1943–44–45)

	Herds tested 10 or more yr. in D.H.I.A.	Non-D.H.I.A. herds	F[a] values
Items used in pairing a D.H.I.A. herd with a non-D.H.I.A. herd			
Crop returns/acre	$ 42.46	$ 43.12	0.14
Labor costs/cow	$ 142.00	$ 140.00	0.05
Number cows milked	24.6	24.1	0.01
Items showing a significant difference			
Rate earned on investment (%)	15.63	11.01	12.18**
Net earnings/acre	$ 29.32	$ 20.93	8.26**
Operator's earnings	$5,187.00	$3,535.00	4.03*
Butterfat produced/cow (*lb.*)	322	268	10.92**
Milk produced/cow (*lb.*)	8,720	7,214	8.72*
Returns above feed cost/cow	$ 295.00	$ 259.00	4.96*
Items not showing a significant difference			
Returns for $100 feed fed to dairy cattle	$ 181.00	$ 163.00	3.63
Size of farm (acres)	208	210	0.00
Machinery costs/acre	$ 13.35	$ 14.29	0.29
Labor costs/acre	$ 21.13	$ 22.83	0.64
Building costs/acre	$ 4.31	$ 4.08	0.08
Returns from hogs	$3,932.00	$3,253.00	0.68
Feed cost/cow	$ 166.00	$ 160.00	0.38
% of land in legumes	32.7	29.9	.80
No. of herds	14	14	

** Significant at the 1 per cent level.
* Significant at the 5 per cent level.
[a] Values obtained by analysis of variance.

same facts. However, in this latter sample when measured statistically, returns for $100 spent for feed fed to the cows on the farms and the feed costs per cow were not significantly different for the paired groups of farms, though these items tended in the same numerical direction as they did in the first sample.

SUMMARY AND CONCLUSIONS

Financial and production records from dairy farms in northern Illinois were used as the source of data to determine the difference in farm operating efficiency between non-D.H.I.A. farms and farms on which D.H.I.A. testing had been done for ten or more years. Rate of interest earned on investment and net earnings per acre were used as the measures of operating efficiency.

Farms on which the herds had been tested for ten or more years in D.H.I.A. had a rate earned on total investment of 14.74 per cent and a net earnings per acre of $30.12. Non-D.H.I.A. farms had a rate earned on investment of 9.72 per cent and a net earnings per acre of $19.11. Much of this difference was due to the fact that the cows in the D.H.I.A. herds had an average butterfat production of 336.9 lb., while the cows in the non-D.H.I.A. herds had an average of only 277.4 lb.

These data support the conclusion that the farms on which the cows had been tested in a Dairy Herd Improvement Association for 10 or more yr. were more efficiently operated than the farms on which no D.H.I.A. testing had been done.

ACKNOWLEDGMENT

The authors express their appreciation to M. L. Mosher of the Agricultural Economics Department, University of Illinois, for his valuable suggestions concerning this study.

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JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

Prepared in cooperation with the
International Association of Ice Cream Manufacturers
and the Milk Industry Foundation

BUTTER

O. F. HUNZIKER, SECTION EDITOR

340. Switch to continuous. Food Eng. Staff. Food Eng., **23,** 4: 98, 99, 177, 179. Apr., 1951.

The operations and advantages of the continuous buttermaking process are described for an installation in an Iowa creamery.

T. J. Claydon

341. Clarifying butterfat. C. E. NORTH. U. S. Patent 2,550,288. 11 claims. Apr. 24, 1951. Official Gaz. U. S. Pat. Office, **645,** 4: 1264. 1951.

In a process for making pure butterfat, the unrefined melted fat, containing about 1% diluted milk serum, is agitated with an acid at a concentration of 0.5% and then allowed to separate by gravity, the fat being further refined by washing with water in a centrifugal separator.

R. Whitaker

342. Plural wire butter cutter. E. H. SARGENT and J. B. WADSWORTH (assignors to Sargent Co.). U. S. Patent 2,550,166. 2 claims. Apr. 24, 1951. Official Gaz. U. S. Pat. Office, **645,** 4: 1236. 1951.

Butter is cut into pats for individual serving by this device, consisting of a series of parallel taut wires. R. Whitaker

CHEESE

A. C. DAHLBERG, SECTION EDITOR

343. Good milk and workmanship produce high quality cheese. H. L. WILSON. Can. Dairy Ice Cream J., **30,** 3: 34–36, 66–68. Mar., 1951.

The author describes milk specifications for cheese manufacture and adequate testing procedures. Details are given on cheese processing with emphasis on the making of cheddar cheese.

H. Pyenson

344. Cheese production. S. ORLA-JENSEN. U. S. Patent 2,549,331. 4 claims. Apr. 17, 1951. Official Gaz. U. S. Pat. Office, **645,** 3: 916. 1951.

Cheese, having Emmenthaler characteristics, is made from milk pasteurized at a high temperature, culturing with *Thermobacterium helveticum* at 40–45° to an acidity of 11° Soxhleit-Henkel, adding rennet, cutting the curd after it has formed and completing the cheese-making operation in the usual manner and curing for 3–4 mo.

R. Whitaker

345. Cheese press. N. R. BURRELL and G. C. THOMPSON (assignors to National Dairy Assoc. of N. Z.). U. S. Patent 2,553,035. 15 claims. May 15, 1951. Official Gaz. U. S. Pat. Office, **646,** 3: 896. 1951.

A cheese press is described which employs a fluid-operated ram, which moves forward and locks as the cheese is compressed. R. Whitaker

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

346. Yogurt buttermilk. G. JOGGARD and M. W. RANSOM, The Dairy Labs., Philadelphia, Pa. Milk Dealer, **40,** 5: 44, 66–71. Feb., 1951.

Suggestions are given as to the methods of pasteurizing milk for manufacture of "Yogurt buttermilk" with the range of practical ripening temperatures and the ideal acidity of 0.80–0.85% to obtain the finest flavor product. The mechanism of the growth of the 3 important bacteria, *S. thermophilus, L. bulgaricus* and *Pl. yogurtii,* and the necessity of controlling the acidity in order to maintain a product with fine flavor and with uniform therapeutic value is explained. The question is raised as to whether Yogurt buttermilk would not be more acceptable than the semi-solid yogurt. C. J. Babcock

347. Yogurt-fermented milk. N. C. ANGEVINE, Meyer-Blanke Co., St. Louis, Mo. Milk Dealer, **40,** 5: 50–51, 62–64. Feb., 1951.

Yogurt is made from a combination of 3 bacteria, namely, *Bacillus bulgaricus, Streptococcus thermophilus* and *Plocaino-bacterium yoghourtii,* the latter merely a specie of *bulgaricus.* With combined cultures of this type, it is necessary to secure fresh laboratory cultures weekly, or at least twice per month. These 3 strains of bacteria must be kept in proper balance and uncontaminated. Directions are given for carrying yogurt cultures and the manufacture of yogurt milk.

C. J. Babcock

348. A study of resazurin reduction in freshly drawn mastitic-like milk. C. A. MCBRIDE and N. S. GOLDING, State College of Wash., Pullman. J. Milk & Food Technol., **14:** 27–30. Jan.–Feb., 1951.

The authors claim the resazurin test will give comparable results with accepted confirmative methods in detecting freshly-drawn quarter samples of mastitic-like milk. A resazurin reading of 3 on a 60-min. incubation period, or 3 on a 90-min. incubation period, is applicable in detecting cases of subclinical mastitis. Leucocyte activity can be measured by the resazurin test on quarter samples of milk within 4 hr. of milking. The dry vial modification of the resazurin test is acceptable as a screening test for mastitis on the farm when properly supervised. H. H. Weiser

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

349. Fat determinations in milk and milk products. L. GERSHENFELD and M. ROSENTHAL, College of Pharmacy, Philadelphia, Penn. J. Milk & Food Technol., **14:** 17–18. Jan.–Feb., 1951.

A rapid and accurate method for fat determinations in milk and milk products was outlined. A mixture of non-ionic detergent, such as Tween, and an anionic detergent, Tergitol, with a fat-soluble dye, Oil Red O, made it possible to obtain a clear and distinct fat column. The results were comparable to the Babcock test, the Schain method and the Gershenfeld and Ucko modification of the Schain procedure.

H. H. Weiser

350. Some azoproteins and their isoelectric points. R. L. MCGEACHIN and B. D. ASHLEY, Univ. of Louisville, Louisville, Ky. J. Am. Chem. Soc., **73,** 3: 1366–1367. Mar., 1951.

The azoprotein formed when casein was coupled with excess diazotized *p*-arsonilic acid was found to have a lower isoelectric point (3.2) than the original protein (4.6).

H. J. Peppler

351. Water absorption of proteins. VI. Effect of guanidino groups in casein. E. F. MELLON, A. H. KORN, E. L. KOKES and S. R. HOOVER, Eastern Reg. Research Lab., U. S. D. A., Philadelphia. J. Am. Chem. Soc., **73,** 4: 1870–1871. Apr., 1951.

Several casein derivatives containing different amounts of substituted guanidino groups were prepared by reacting S-methyl-thiourea in alkaline solution with the free amino groups. The data determined for the vapor phase water absorption isotherms of these samples revealed that the absorption of guanidino groups is not significantly different from the absorption of the free amino groups themselves. H. J. Peppler

352. Spontaneous gelation of alkaline casein dispersions. K. F. PLOMLEY, H. G. HIGGINS and J. F. HAYES. Nature, **167,** 4241: 224. 1951.

A study was made of the gelation of casein in water with NaOH. At pH 12.5 the viscosity increase with time was slow and linear. At pH 13.0 and 13.5 the increase was rapid and nonlinear. When these gelated caseins were brought to the isoelectric point, dialyzed and viscosity studies made in buffer solutions, there was no evidence of unravelling of the polypeptide chains; in fact, the molecular symmetry increases at the high pH levels. H_2S is liberated when gelated casein is neutralized. The exact mode of molecular linkage of casein concerned in the gelation process is not yet understood; it appears to be different from other denatured proteins, such as egg albumin. R. Whitaker

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

353. Apparatus for pasteurizing and the like. D. B. VANDEWATER (assignor to DeLaval Separator Co.). U. S. Patent 2,551,651. 23 claims. May 8, 1951. Official Gaz. U. S. Pat. Office, **646,** 2: 438. 1951.

A heater and a control system for operating a pasteurizer are described. R. Whitaker

354. Milk pasteurization method and apparatus. E. MITTELMANN. U. S. Patent 2,550,584. 5 claims. Apr. 24, 1951. Official Gaz. U. S. Pat. Office, **645,** 4: 1340. 1951.

Milk is continuously preheated by the fluid used to cool the electronic tube of an oscillator used to supply the radio frequency energy to a dielectric heater, which heats the milk to the pasteurization temperature. R. Whitaker

355. Plate-type heat exchanger. H. H. EHRMAN (assignor to York Corp.). U. S. Patent, 2,550,-339. 7 claims. Apr. 24, 1951. Official Gaz. U. S. Pat. Office, **645,** 4: 1277. 1951.

Heating and cooling are facilitated in a plate-type heat exchanger by impressing in the plates indentations and grooves which channel the flow of the liquids between the plates in a manner to provide improved temperature distribution.

R. Whitaker

356. Method and apparatus for pasteurizing liquids. F. L. STEGHART (assignor to Tinsley, Ltd.). U. S. Patent 2,549,342. 6 claims. Apr. 17, 1951. Official Gaz. U. S. Pat. Office, **645,** 3: 191. 1951.

An electrical control system for maintaining the desired temperature going to the holding tube of an HTST pasteurizer is described.

R. Whitaker

357. Apparatus and method for preserving products in sealed containers. W. MCK. MARTIN (assignor to James Dole Eng. Co.). U. S. Patent 2,549,216. 7 claims. Apr. 17, 1951. Official Gaz. U. S. Pat. Office, **645,** 3: 885. 1951.

Foods, such as evaporated milk, are sterilized continuously in bulk and aseptically canned in this device into sterile containers. The cans and covers are continuously sterilized by flowing superheated steam and are filled with the sterile product at atmospheric pressure.

R. Whitaker

358. Unit heaters in the dairy industry. Anonymous. Milk Dealer, **40,** 5: 45, 72–74. Feb., 1951.

Unit heaters are classified as the propeller-fan

type and the centrifugal-fan type. Either type may be designed to blow the air horizontally or vertically. They can handle simple drying problems effectively. They provide a practical and economical means of heating milk houses and can be used effectively for drying trucks after washing. In addition to their utilitarian functions in industrial processes, unit heaters provide maximum physical comfort. They heat quickly from a cold start and maintain the desired temperature, providing a flexible heating system with considerable savings in fuel costs. In purchasing unit heaters care should be taken to determine that they are rated in accordance with the standard test code adopted jointly by the Industrial Heater Association and the American Society of Heating and Ventilating Engineers. C. J. Babcock

Also see abs. no. 340, 345, 373.

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

359. Pallet system of materials handling. L. H. HECKENDORN, Heckendorn Bros., Havertown, Pa. Milk Dealer, **40,** 5: 42–43, 71. Feb., 1951.

Actual analysis and experience show this system to be worthwhile in plants operating as few as 20 routes, and in new or remodeled plants milk dealers operating less than 20 routes often can make remarkable savings. Practically every other industry in America has adopted this system in whole or in part. The advantages of the system are: (a) Considerably less labor is required in day and night cold box operations, empty bottle handling, checking in routes, handling supplies and other products, loading and unloading delivery trucks, etc. Labor can be reduced 25–75%. (b) Better inventory control of the empty box and bottles returned to the plant. (c) Less inventory loss of finished products in loading out operation. (d) Greatly increased life of glass bottles. (e) Increased life of cases by handling them less frequently and more gently. (f) Eliminating up to 2 of the 3 individual handlings of each filled case of milk per day, considerably reducing the milk losses caused by breaking filled glass bottles; also, breakage of empty bottles in cases is reduced. (g) Requires very little extra labor to load and unload the delivery trucks for drivers. (h) The hour a day or more required per driver to load and unload and wait in line is reduced to a few minutes per day. (i) Eliminates the hard work of loading individual cases of milk into delivery trucks.

C. J. Babcock

360. Court decisions on ordinances which restrict a market. F. MEISSNER. Milk Dealer, **40,** 5: 46, 64–66. Feb., 1951.

The following court cases are discussed: Higgins vs. City of Galesburg; Dean Milk Co. vs. City of Waukegan; and La Franchi vs. Santa Rosa. The following conclusions are drawn: (a) The primary purpose of city ordinances and grade A laws is to insure a sufficient supply of wholesome milk to consumers in all communities. The objective should, therefore, not be interpreted as a confinement of marketing of milk in general or limitations of any particular milk to any particular market. If reasonable regulations properly applied result in limitations, then it must be accepted as a necessary consequence of the effort to protect the health of citizens. (b) Milk in cartons usually meets the requirements to the same degree as milk in glass bottles. The court decisions seem unanimously to decry the use of health ordinances as a device to monopolize or restrict trade. They point out the desirability of instituting uniform and adequate state inspection outside corporate limits, furthering the establishment of a free flow of milk. C. J. Babcock

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

361. Efficiency of food utilization for milk production. J. E. NICHOLS. Nature, **167,** 4250: 610. 1951.

Cows can be made more efficient if food recording can be conducted along with milk volume and butterfat content and the information used in determining the best lactating period and frequency of calving. R. Whitaker

362. Hydrogenation of polyunsaturated fatty acids by the ruminant. R. REISER. Fed. Proc., **10,** 1 (Part 1): 236. 1951.

Very little linolenic acid is deposited in the body fats of sheep and cattle, even where rations high in this acid are fed. Nonruminants (horses) deposit appreciable amounts under the same conditions. The hypothesis that hydrogenation of linolenic acid occurs in the rumen as a result of microbiologcal action was tested. Four-day incubation of rumen contents (linseed oil mixture) revealed that linolenic acid was reduced from 30 to 5% and linoleic acid increased proportionately but other acids remained unchanged. No hydrogenation occurred in autoclaved controls.

S. Patton

363. A proposed modification of the A.O.A.C. method for carotene in alfalfa. C. R. THOMPSON and E. M. BECKOFF, Western Reg. Research Lab., Albany, Cal. J. Assoc. Off. Agr. Chemists, **34,** 1: 219–224. 1951.

The present A.O.A.C. method for carotene in plant materials contained several sources of error. An insufficient amount of eluant and variation in the degree of adsorptive power of the magnesia used in the chromatographic adsorption contributed to incomplete elution of the carotene and consequent low carotene assays. Loss of acetone during refluxing caused increased retention of carotene. A procedure is outlined which eliminated these sources of error. The new method also is simpler, more rapid and better suited to routine assays. F. J. Babel

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

364. Grazing behavior of dairy cattle in the tropics. W. J. A. PAYNE, W. I. LAING and E. N. RAIVOKA. Nature, **167,** 4250: 610. 1951.

Cows transported from temperate climates to the tropics change their grazing habits. For best results the technic of herd management should be arranged to recognize these differences and technics used in temperate areas should not be followed. Shady paddocks should be provided for daytime grazing and the best pasture should be reserved for night and early morning grazing.
R. Whitaker

365. Alarm device for milking machines. A. G. PERKINS. U. S. Patent 2,549,231. 12 claims. Apr. 17, 1951. Official Gaz. U. S. Pat. Office, **645,** 3: 889. 1951.

An alarm is operated when the milk flow entering a milker reservoir diminishes to a predetermined minimum at the end of the milking process. R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

366. Ice cream mix design and calculation. J. N. LUCKIE, Foremost Dairies, Inc., Oakmont, Pa. Ice Cream Trade J., **47,** 1: 48, 49. Jan., 1951.

State laws usually specify the minimum percentages of butter fat, total solids and ingredients which may be used. Some states have overrun standards. Consumer preference, cost and proper food balance indicate a fat content of 10–12% as ideal; however, premium ice cream may contain up to 18–22% fat. Although milk-solids-not-fat add to the body and smoothness of texture, the amount used may be limited because of sandiness and cooked flavor. After a formula has been selected, the amount of ingredients to use may be calculated using the serum point method. An example of how these calculations are made is presented. W. H. Martin

367. Controlled automatic fluid proportioning in ice cream mix making. R. K. LAWTON, Abbots Dairies, Inc., Philadelphia, Pa. Ice Cream Trade J., **47,** 2: 36. Feb., 1951.

Production efficiencies start with the handling of mix ingredients. Abbots have adopted a system of using 80% frozen cream, condensed milk, liquid sugar (a cane and corn blend) and fluid milk. The condensed milk and liquid sugar are handled in bulk tanks. Flowrator units whose accuracy is between 1 and 2% are used to measure ingredients. A machine is used for incorporating 80% fat-frozen cream directly into flowing warm mix. A 40,000 lb./hr. Ste-Vac heater is used. A changeover to H.T.S.T. pasteurization will make it possible to increase the volume of mix processed per hour. W. H. Martin

368. Frozen confections. L. H. BURT (assignor to Hercules Powder Co.). U. S. Patent 2,548,865. 5 claims. Apr. 17, 1951. Official Gaz. U. S. Pat. Office, **645,** 3: 792. 1951.

A carboxyalkyl carbohydrate is used for stabilizing ice cream and other frozen confections at the rate of 0.01–0.5% to improve whipping time, body and texture. R. Whitaker

369. How to meet problems in fruit and nut ice cream. W. R. KISER, Sou. Dairies, Inc., Wilson, N. C. Ice Cream Trade J., **47,** 4: 42, 43, 58. Apr., 1951.

Frozen strawberries may be purchased from the packer or fresh berries may be purchased and packed by the ice cream manufacturer. When possible, berries should be examined at the packer's warehouse to make sure that no unsound, green, and over-ripe berries are used and to see that they are handled in a sanitary manner. The berries should be free from foreign objects. If it is not possible to visit the packing plant, the fruit should be carefully examined when it enters the ice cream plant. Three parts of berries to 1 part of sugar is the standard pack and the addition of a fruit stabilizer is desirable.

If berries are packed at the ice cream plant, they should be examined for blemishes or inferior berries, washed, capped and sliced. Consumer preference studies indicate that ice cream should contain about 25% quartered berries. Stabilized berries have a more desirable red color, firmer appearance than unstabilized berries and result in ice cream which has a good flavor and better texture.

The prepared berries should be stored at 0 to −20° F. until ready to use and then allowed to thaw just overnight. All utensils coming in contact with the berries should be sanitized to prevent bacterial contamination. After mixing, the berries should be strained and the juice added to the mix in the mix tank just prior to freezing. The berries should be injected into the ice cream through a fruit feeder and the packaged ice cream placed in the hardening room promptly.

Cold-packed bananas are now available and preferred to fresh bananas due to the convenience in their use.

Frozen fruit juices for sherbets and ices are recommended when they are available.

The same sanitary precautions should be used in handling nuts; nuts should be quartered rather than ground into a fine pulp. Care should be taken to prevent nuts from warming up the ice cream and the packages should be placed in the hardening room and hardened quickly for a smooth textured ice cream. W. H. Martin

370. Ice cream manufacture. J. W. KNECHTGES (assignor to Swift and Co.). U. S. Patent 2,550,656. 6 claims. Apr. 24, 1951. Official Gaz. U. S. Pat. Office, **645,** 4: 1358. 1951.

Iciness in the fruit used in ice cream is prevented by separating the juice from the pulp of sweetened fruit, stabilizing the juice with a gel-forming substance and recombining with the pulp so that the pulp and juice mixture sets to a gel before its use in ice cream. R. Whitaker

371. Application of Vacreator and Mallorizer for H.T.S.T. heating of ice cream mixes. P. H. TRACY, J. TOBIAS and E. O. ANDERSON, Univ. of Ill., Urbana. Ice Cream Trade J., **47,** 4: 76, 79, 105. Apr., 1951.

Satisfactory pasteurization of the ice cream mix

was obtained when the Vacreator was operated so that a minimum temperature of 194° F. was maintained in the 1st chamber and a temperature of 185° F. was considered satisfactory when the Mallorizer was used. W. H. Martin

372. Overrun considerations in making ice cream. C. W. ENGLAND, High's Dairy Prod. Co., Washington, D. C. Ice Cream Trade J., **47,** 4: 48, 92. Apr., 1951.

The factors which regulate overrun are type of package, legal regulations on weight per gallon, price, type of flavor and solids content. The usual range varies from 45% for the "bulk type" carry-home package to a maximum of 90–100% for the bulk ice cream.

The factors which affect overrun are percentage composition of the mix, ingredients composition of the mix, source of fat, type of stabilizer, presence of emulsifiers, efficiency of homogenization, design and speed of the freezer and condition of freezer blades.

Overrun percentages may be calculated either by volume or weight. Illustrations are given. In figuring overrun of fruit or ice cream containing bulky flavors, these materials should be taken into consideration. W. H. Martin

373. Pertinent production problems. C. H. MINSTER, Greenbrier Dairy Prod. Co., Beckley, W. Va. Ice Cream Trade J., **47,** 3: 72, 73, **96.** Mar., 1951.

How to maintain the desirable qualities of ice cream until the product reaches the consumer is an important problem facing all ice cream manufacturers. Education of plant and store personnel is essential in the solution of this problem.

Another problem affecting operation efficiency and economy is coordinating the equipment for the most economical operation. The refrigeration load and the size of the evaporators should be adjusted so that compressors may be operated at the maximum possible suction pressure.

W. H. Martin

374. Control through marking ice cream cartons. B. A. BEANE, The Borden Co., Pittsburgh, Pa. Ice Cream Trade J., **47,** 4: 34, 97, 98. Apr., 1951.

To insure neat legible flavor designation on all packages of ice cream, a no. 240 Multigraph printer is used. The machine may be operated as a hand- or an automatic-fed unit. By using the automatic feed, 5,400 cartons/hr. are stamped on 4 sides in a single operation. The machine costs about $1,300 for the complete unit and makes it possible to have a flavor-stamped carton which will not blur, resulting in a happy route salesman, an appreciative sales department and a satisfied customer. W. H. Martin

375. Square bulk containers. Anonymous. Ice Cream Trade J., **47,** 1: 26, 27, 82. Jan., 1951.

The Miller Dairy Farms of Eaton Rapids, Mich., is using a 3.5-gal. disposable, square container for bulk ice cream. Increased capacity of hardening room and dealer cabinets has resulted. The containers are stacked so that air will circulate around them. The containers are 8⅞″ on all sides and are made of 275 lb. unbleached kraft with a special wax-impregnated lining of bleached kraft. A special base board of the same material as the liner is inserted before filling to eliminate excess pressure on the interlocked base flaps.

W. H. Martin

376. Apparatus for filling containers with ice cream or the like. F. C. GROSS (assignor to Package Machinery Co.). U. S. Patent 2,553,-250. 15 claims. May 15, 1951. Official Gaz. U. S. Pat. Office, **646,** 3: 954. 1951.

An automatic machine which fills cartons with ice cream from alternately operated filling spouts into cartons propelled to the 2 filling positions by a pair of conveyors is described. R. Whitaker

377. A report on open top ice cream cabinets. Anonymous. Ice Cream Trade J., **47,** 1: 24, 25, 84. Jan., 1951.

The results of a survey made by the International Association of Ice Cream Manufacturers on cabinet preference show the following: An overwhelming percentage believe that open-top cabinets increase ice cream sales; the most popular sizes were 8-, 12- and 10-ft. cabinets; open-top cabinets are used primarily for display with supplementary storage; the glass-front type of cabinet was preferred provided it was available at a reasonable price; and preference was expressed for illuminated ice cream pictures for merchandising purposes, rather than mirrors for reflecting the actual product. W. H. Martin

378. Apparatus for making frozen confections. C. MCCARL. U. S. Patent 2,549,915. 4 claims. Apr. 24, 1951. Official Gaz. U. S. Pat. Office, **645,** 4: 1168. 1951.

Structural features are given covering a mold and stick holder for making frozen confections in a brine tank. R. Whitaker

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

379. The expansion of the cream volume of fluid milk by the addition of superheated condensed milk and its detection. A. C. SMITH and F. J. DOAN, Pennsylvania Agr. Expt. Sta., State College. J. Milk & Food Technol., **14:** 23–26, 45. Jan.–Feb., 1951.

A comparison of the fat content of the cream layers of 52 different herd milks varied from 22.45–28.73%, with an average of 25.55%. Adulterated milks were not as high as the lowest values of the normal cream layers. Any value below 22% fat is suspicious of this form of adulteration.

Typical mastitic milk, when flaky, will expand the cream layer when added to normal milk.

Three significant criteria are: (a) If the ratio of fat to casein in the cream layer is less than 10, it is evidence of adulteration; (b) if the ratio of fat to solids-not-fat in the cream layer is less than 3, adulteration is suggested; (c) if the fat content

of the cream layer is lower than 22%, adulteration may be involved. H. H. Weiser

380. Sediment tester filter disc restrainer. R. E. Quinn (assignor to Langsenkamp-Wheeler Brass Works). U. S. Patent 2,553,472. 5 claims. May 15, 1951. Official Gaz. U. S. Pat. Office, **646,** 3: 1011. 1951.

Structural details are given for a device for holding a filter disc or pad in a sediment tester. R. Whitaker

381. Milk bottle holder. E. M. Navarro. U. S. Patent 2,549,510. 2 claims. Apr. 17, 1951. Official Gaz. U. S. Pat. Office, **645,** 3: 963. 1951.

A device for locking and protecting the top of milk bottles on a consumer's doorstep is described. R. Whitaker

382. Valve-spout cream remover. J. A. Danielson. U. S. Patent 2, 552,154. 1 claim. May 8, 1951. Official Gaz. U. S. Pat. Office, **646,** 2: 573. 1951; and **Liquid-dispensing valve spill.** J. A. Danielson. U. S. Patent 2,552,155. 2 claims. May 8, 1951. Official Gaz. U. S. Pat. Office, **646,** 2: 574. 1951.

A device for inserting into the side of paper containers of cream-line milk for withdrawing the side the container open on top and the portion cream consists of a pointed tube with portion in-outside bent slightly downward to facilitate directing the flow into a receiving vessel. R. Whitaker

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. Jenness, Section Editor

383. Heat processing and nutritive value of milk and milk products. L. J. Schroeder, M. Iacobellis and A. H. Smith. Fed. Proc., **10,** 1 (Part 1): 393. 1951.

Heat processing as used commercially in the preparation of evaporated milk and dry whole milk did not decrease the nutritive value of the proteins according to these experiments. The milk or milk products were used as the sole source of protein in an otherwise purified diet. Nitrogen balances in 4 adult female dogs were used as criteria of change in the milk proteins as a result of processing. S. Patton

384. Preference for methionine-supplemented casein (versus unsupplemented casein) by rats on a self-selection regime. E. C. Albritton, L. P. Munan and H. L. McCorkle. Fed. Proc., **10,** 1 (Part 1): 4. 1951.

Three strains of rats all consumed significantly greater quantities of casein supplemented with methionine as compared with unsupplemented casein. S. Patton

385. Supplemental value of cystine and methionine for low protein (casein) diets. H. B. Lewis and R. S. Fajan. Fed Proc., **10,** 1 (Part 1): 387. 1951.

Experiments with rats demonstrated that in the presence of sufficient choline, the amounts of sulfur-containing amino acids, particularly methionine, effective in the promotion of growth may be much less than previously estimated. S. Patton

SANITATION AND CLEANSING

K. G. Weckel, Section Editor

386. The sanitizing of milk cans in mechanical can washers. S. L. Tuckey, G. W. Reinbold, P. H. Tracy and R. V. Hussong, Univ. of Ill., Urbana. J. Milk & Food Technol., **14:** 7–41. Jan.–Feb., 1951.

Part 1 is a survey on milk can washing operations in 13 milk plants, involving 304 cans. 54% of the cans had a count of 40,000 or less. Physical condition of the cans was not an accurate index of the bacteriological condition. 42% of the cans which were free of corrosion and milk stone had counts of more than 40,000/can.

Part 2 was a study of the factors influencing the final bacterial count in a milk can. When the temperature of the wash solution was maintained at 140° F., 73% of the bacterical counts on the cans were 40,000 or less; at 150° F., 91% of the counts were below this figure; and at 160° F., 96% of the cans met the standard of 40,000 or less.

Part 3 involved the use of chemical sterilizers. Without chlorine, 38% of the cans met the standard of 40,000 or less per can. When 30 ml. of 500–750 ppm. of chlorine were injected into cans by the first steam jet on a straight-way washer, 78% of the cans met the standard. The effect of the chlorine was lost after 48 hr. Similar results were obtained when a quaternary ammonium compound was added to the wash solution. No reduction in the bacterial count was noted after 60 min. of use. H. H. Weiser

387. The control of microorganisms populations. A. L. Sotier, Wyandotte Chemicals Corp. Milk Dealer, **40,** 5: 47, 56–58. Feb., 1951.

A discussion of the quaternary ammonium and chlorine types of detergent-sanitizers and their advantages is summarized as follows: Germicides are being improved constantly, their zones of usefulness are being extended and better defined, while new ones are being discovered. Certainly the vast majority of commercial products have a rightful place in the field of practical use. One type may give better performance on one class of work, while a different type excels on another class. The user of germicides should learn all that he can of the different products, especially their advantages and limitations for the specific job in mind. He should exercise good common sense when he buys and uses germicides. No germicide is a magic cure-all, and none will substitute for good housekeeping, thorough washing or the exercise of intelligence. C. J. Babcock

FILTER FACTS FOR DAIRY SANITARIANS

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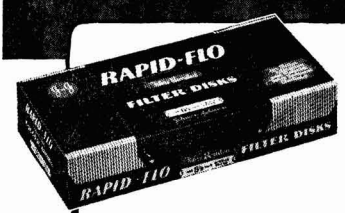
all 3 are *Fibre-Bonded*

Each has its purpose. No One Disk can serve the Function of all 3!



All 3 Rapid-Flo FIBRE BONDED Filter Disks provide Safer Filtration and a more reliable Rapid-Flo Farm Sediment Check-Up . . . the producers own regular test to aid Quality Milk Production and give *extra* protection against loss of milk due to sediment.

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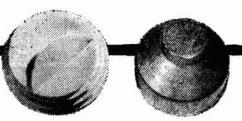
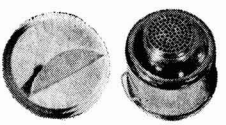
Dairy technicians, sanitarians, field men and leading health authorities know these facts. It is to the dairy industry's best interest to urge regular use of safer, more retentive Improved Formula Rapid-Flo Fibre-Bonded Filter Disks.

J & J gauze facing on one or both sides of J & J Fibre-Bonded Disks provides the extra safety, necessary for some types of strainers.



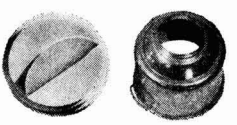
TYPE 1

WITHOUT GAUZE FACING—May safely be used in *most* metal strainers that have a baffle (plate, cup or dome) above the disk and support below, where slots or holes are less than ¼" in diameter.



TYPE 2

SINGLE FACED—GAUZE ON ONE SIDE—Gauze *supports* the disk, where metal strainer lacks such support, when slots or holes are ¼" in diameter or larger.



TYPE 3

DOUBLE FACED—GAUZE ON BOTH SIDES—Gauze protects and supports disk when such support and protection is lacking in the form of "open-bottom" strainers.

FILTER PRODUCTS DIVISION **Johnson & Johnson** 4949 WEST 65th STREET CHICAGO 38, ILLINOIS