JOURNAL OF DAIRY SCIENCE

Contents

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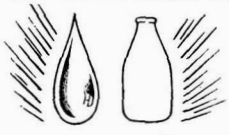

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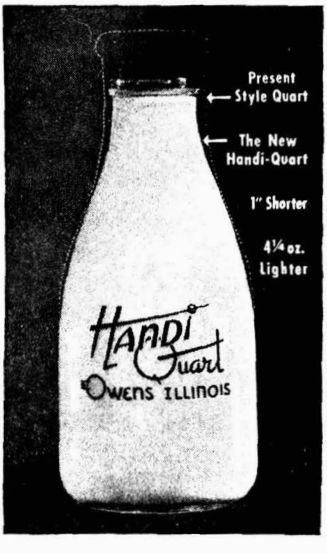

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ERRATA

Vol. 25

Page 635: *The fourth paragraph should read* The calf seems to be able to utilize rather diverse mixtures of plant products. In the popular bulletin of Herman (105) are recommended mixtures varying from 30 to 50 per cent in corn meal, from 25 to 30 in ground oats, from 20 to 30 per cent in wheat bran and from 10 to 25 per cent in linseed oil meal.

Page 648: *The first reference should read* (183) MORRISON, F. B., HULCE, R. S., AND HUMPHREY, G. C. Wis. Agr. Expt. Sta. Bul. 339, p. 134. 1922.

Page 648: *The second reference should read* (184) MORRISON, F. B., HULCE, R. S., AND HUMPHREY, G. C. Wis. Agr. Expt. Sta. Bul. 362, p. 96–99. 1924.

Page 785: *Authors' names should read:* NORMAN L. JACOBSON, DWIGHT ESPE AND C. Y. CANNON.

Page A346: Line 22 should read SCALES, F. M., better cleaning, A74.

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THE ROLE OF OXIDASE-PRODUCING BACTERIA IN THE DEVELOPMENT OF OXIDIZED FLAVOR IN MILK

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INTRODUCTION

Gram-negative oxidase-producing bacteria commonly occur in milk. Many of them are capable of growing at relatively low temperatures, and they frequently become a serious problem in the preparation and storage of certain dairy products. Castell and Garrard (1) and Castell (2) have studied these organisms especially in their relation to the spoilage of butter and cheese. No data have been reported which show the relationship of these organisms to oxidized flavor in milk. The speculation, however, has been made from time to time that such a relationship might exist. The work reported here was planned to determine whether or not the oxidase-producing organisms can cause the development of oxidized flavor in milk.

PROCEDURE

Nine cultures of Gram-negative rods, probably representing 7 species, were used in these experiments. All gave a strongly positive reaction for oxidase with p-aminodimethylaniline monohydrochloride. The cultures were identified by number as follows:

No. 1. *Pseudomonas putrefaciens,* obtained from Iowa State College.

No. 2. *Pseudomonas fragi,* obtained from Iowa State College.

Nos. 4 and 5. A *Pseudomonas* species isolated from pasteurized milk.

Nos. 6, 7, 8, 9, and 10. Unidentified, isolated from pasteurized milk.

Cultures used for inoculation were grown overnight at 20°–22° C. in sterilized milk. Just before using the culture a rough estimate of the number of the organisms it contained was made microscopically as a basis for calculating the size of the inoculum.

In each experiment the milk used was from cows known, as the result of prior testing, to produce milk which was relatively susceptible to the development of oxidized flavor. The milk was pasteurized in flasks in a water bath at 63° C. for 30 minutes, and then cooled to a temperature of 10°–15° C. The milk was distributed in smaller lots into sterilized flasks for inocu-

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lation and addition of copper. As each lot was completed it was bottled in sterile half-pint bottles, capped, and placed in a household refrigerator at 4°–6° C.

Examination of each lot was made immediately and again after 2 days and 4 days in the refrigerator. A previously unopened bottle was used for each examination. The samples were plated on standard TGEM agar. The plates were incubated at 20°–25° C. for 4 days. Total counts were made on the incubated plates, after which the plates were flooded with a freshly prepared 0.5 per cent aqueous solution of p-aminodimethylaniline monohydrochloride and an estimate was made of the number of oxidase-positive colonies.

The portion of the sample remaining after plating was tasted for the presence of oxidized flavor. The judging was done without knowledge of the previous treatment of the sample. The flavor results were recorded as numbers so that averages could be taken in the analysis of the data. Absence of oxidized flavor was recorded as 0, and when present the flavor was scored as 1, 2, 3, or 4, in the order of increasing intensity.

EXPERIMENTAL

In the first part of this work milk was used which would not develop oxidized flavor spontaneously but would develop an intense oxidized flavor with the addition of as little as 0.1 p.p.m. of copper. In most of the experiments, inoculations with the test organisms were made into milk both with and without added copper. Four degrees of inoculation were used: Light (L), varying from less than 100 to 19,900 of the test organisms per ml. of the freshly inoculated milk; moderate (M), 20,000 to 99,000 organisms per ml.; heavy (H), 100,000 to 1,000,000 organisms per ml.; and very heavy (VH), more than 1,000,000 organisms per ml. The results of this work are shown in table 1.

No sample to which copper was not added developed an oxidized flavor during a 4-day storage period. This was true both with the inoculated samples and the uninoculated controls. All uninoculated controls to which 0.05 or 0.1 p.p.m. of copper was added developed an oxidized flavor. Likewise, all copper-contaminated samples with light or moderate inoculations developed an oxidized flavor, but in no inoculated sample was the flavor intensity greater, at the end of 4 days, than it was in the corresponding uninoculated control. In all but one group of samples with added copper, the flavor intensity appeared to be less in inoculated samples. When heavy or very heavy inoculations were employed, the average flavor intensity was usually distinctly less than that of the corresponding controls. Some of the samples with 0.1 p.p.m. of added copper were completely protected by these heavy inoculations from becoming oxidized.

Work was also done to determine whether or not the test organisms in

TABLE 1

The effect of inoculation of oxidase-positive bacteria into milk susceptible to the development of oxidized flavor upon the addition of 0.1 p.p.m. of copper

Culture No.	Grade of inoculation	No. of experiments	Average of inoculations	Intensity of oxidized flavor				Total counts after 4 days at 4°–6° C. (average)	
				Inoculated sample		Uninoculated control			
				2 days	4 days	2 days	4 days	Inoculated sample	Uninoculated control
Without copper added									
1	L*	5	5,240	0	0	0	0	35,400	4,550
1	M	3	49,000	0	0	0	0	1,190,000	2,440
1	H	1	110,000	0	0	0	0	1,760,000	10,900
2	L	2	13,000	0	0	0	0	9,800,000	10,900
2	H	2	316,000	0	0	0	0	23,500,000	10,900
2	VH	2	5,830,000	0	0	0	0	38,000,000	10,900
4 and 5	L	2	5,700	0	0	0	0	10,250,000	15,700
4 and 5	M	1	94,500	0	0	0	0	11,500,000	14,000
4 and 5	VH	3	2,700,000	0	0	0	0	18,900,000	14,000
6	L	1	4,600	0	0	0†	0	28,000	17,300
7	L	1	2,400	0	0	0†	0	19,800	17,300
8	L	1	9,500	0	0	0†	0	21,700	17,300
9 and 10	L	2	6,000	0	0	0†	0	54,000	17,300
Mixed 1 to 10	L	2	10,400	0	0	0†	0	7,700,000	17,300
With 0.1 p.p.m. of added copper									
1	L	4	5,520	1.75	2.75	1.75	3.0	18,100	7,130
1	M	2	56,000	0.0	2.0	0.5	2.0	508,000	3,360
1	H	1	110,000	3.0	3.0	3.0	4.0	2,020,000	9,900
2	L	2	13,000	2.5	3.0	3.0	3.5	2,675,000	7,390
2	H	2	316,000	0.0	0.0	3.0	3.5	12,500,000	7,390
2	VH	2	5,830,000	0.0	0.0	3.0	3.5	25,500,000	7,390
4 and 5	L	1	3,500	1.0	2.0	2.0	4.0	4,600,000	10,000
4 and 5	M	1	94,500	1.0	2.0	2.0	4.0	22,800,000	10,000
4 and 5	VH	1	2,700,000	0.0	0.0	2.0	4.0	25,000,000	10,000

* L = < 20,000; M = 20,000 to 99,000; H = 100,000 to 1,000,000; VH = > 1,000,000.
† Control with 0.05 p.p.m. of copper developed oxidized flavor of 4 within 2 days.

TABLE 2

The effect of inoculating oxidase-positive bacteria into milk capable of developing oxidized flavor spontaneously

Culture No.	Grade of inoculation	No. of experiments	Average of inoculations	Intensity of oxidized flavor				Total counts after 4 days at 4°–6° C. (average)	
				Inoculated sample		Uninoculated control			
				2 days	4 days	2 days	4 days	Inoculated sample	Uninoculated control
1	H*	1	222,000	2.0	2.0	1.0	4.0	3,500,000	700
1	VH	2	4,465,000	0.0	0.0	1.5	4.0	12,000,000	660
2	H	1	145,000	0.0	0.0	1.0	4.0	9,800,000	700
2	VH	2	4,235,000	1.0	0.0	1.5	4.0	16,250,000	660
4 and 5	VH	1	3,700,000	0.0	0.0	2.0	4.0	26,000,000	610
6	VH	1	3,100,000	0.0	0.0	2.0	4.0	6,400,000	610
8	H	1	790,000	2.0	3.0	2.0	4.0	490,000	610
9	VH	1	1,240,000	1.0	2.0	2.0	4.0	5,900,000	610
Mixed 1, 2, 4, 6, 8 and 9	VH	1	3,000,000	0.0	0.0	2.0	4.0	40,000,000	610

* H = 100,000 to 1,000,000; VH = > 1,000,000.

massive inoculations were capable of inhibiting the development of oxidized flavor in milk in which the flavor developed spontaneously without added copper. The results are shown in table 2.

All of the uninoculated controls became oxidized with a flavor intensity of 4. Only 3 of the 11 inoculated samples became oxidized during the 4-day storage period and in none of these samples was the flavor as intense as it was in the corresponding uninoculated control. The flavor developed in only one sample in which the inoculation was as great as 1,000,000 per ml. of milk. One sample which received an inoculation of only 145,000 per ml. of milk did not become oxidized, but in this sample the culture grew rapidly to reach a count of 9,800,000 in 4 days.

DISCUSSION

In these experiments nine strains of oxidase-producing organisms which probably represent seven different species were used. There were three species of the genus *Pseudomonas* represented by four strains. The remaining five strains were Gram-negative rods that probably belong to the genus *Alcaligenes* or the genus *Achromobacter*. None of the five attacked glucose or lactose, but instead developed an alkaline reaction in the sugar broths. All produced an alkaline reaction in litmus milk and one slowly digested the milk. Other characteristics were variable and definitely separated all but two of the five strains.

Although there probably are many species of oxidase-producing bacteria that may gain access to milk, we believe that the group of cultures used is sufficiently representative to show that these organisms do not contribute to the development of oxidized flavor in pasteurized milk. None of these cultures showed such a tendency, but on the contrary appeared to inhibit the development of oxidized flavor even in some of the samples with only light or moderately heavy inoculations. This inhibition of the development of oxidized flavor appeared to depend partly on the amount of the inoculation and partly on the rapidity with which the organisms grew under the storage conditions. Evidence is completely lacking that these organisms contribute to the development of oxidized flavor. They appear not to differ greatly from other milk organisms with respect to their influence on this flavor defect.

SUMMARY AND CONCLUSIONS

Nine cultures of oxidase-producing Gram-negative bacteria were inoculated into freshly pasteurized milk that was susceptible to the development of oxidized flavor either spontaneously or with the addition of no more than 0.1 p.p.m. of copper. The amount of inoculation was varied from less than 100 to several million organisms per ml. of inoculated milk. These inoculated samples and suitable controls were stored at 4°–6° C. for 4 days.

No inoculated sample developed a greater intensity of oxidized flavor than that of the corresponding uninoculated control. When large inoculations were used the development of oxidized flavor was markedly or completely inhibited.

Evidence is completely lacking that the oxidase-producing bacteria differ greatly with respect to oxidized flavor in milk from other bacteria commonly found in milk.

REFERENCES

(1) CASTELL, C. H., AND GARRARD, E. H. "Oxidase Reaction" of Bacteria in Relation to Dairy Products. Food Res., **5**: 2, 215–222. 1940.

(2) CASTELL, C. H. Oxidase-Positive Bacteria in Dairy Products. Canad. Dairy and Ice Cream Jour., **19**: 5, 26–28, 42. May, 1940.

REDUCED AND TOTAL VITAMIN C IN MILK

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INTRODUCTION

Simple titration of milk with 2,6-dichlorphenolindophenol measures the content of reduced ascorbic acid but does not measure the total vitamin C activity of the milk if dehydroascorbic acid is present. In the presence of oxygen the reduced ascorbic acid in ordinary milk is oxidized slowly to dehydroascorbic acid, which, in turn, slowly disappears because of its own instability. The relative amounts of these two forms of ascorbic acid will depend on the age of the milk, previous history, exposure to light, copper content, and temperature of storage. It is the purpose of this paper to report the range of concentrations of dehydroascorbic acid which are normally encountered in milk and to show under what conditions the simple titration for reduced ascorbic acid can be used as a measure of total vitamin C activity.

Determinations of total vitamin C have been made by reducing any dehydroascorbic acid present by treatment with a suspension of *Bacterium coli* (3) prior to titrating with 2,6-dichlorphenolindophenol (8).

EXPERIMENTAL

Immediately after milking, all of the vitamin C is in the form of reduced ascorbic acid. Table 1 shows analyses of milk from individual cows taken directly in brown bottles and titrated immediately. These results confirm

TABLE 1

Ascorbic acid and total vitamin C in milk at time of milking (individual samples milked into brown bottles)

Reduced ascorbic acid	Total vitamin C
mg./l.	*mg./l.*
25.1	24.4
30.9	29.5
23.8	25.1
22.7	23.8
28.5	26.8
24.4	24.1
25.1	25.5
21.0	20.7
18.7	20.4
26.1	25.5
22.4	23.1
Ave. 23.4	23.5

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the findings of Kon and Watson (7) which were based on the use of the hydrogen sulfide method of reducing dehydroascorbic acid.

Analyses were made on 12 samples of commercial milk taken from cans at the time of arrival at the country plant and stored at 1° C. At the beginning of storage the relative amount of dehydroascorbic acid increases. Between the first and sixth days of storage, the dehydroascorbic acid remains fairly constant, between 3 and 6 mg. per liter. The results of the individual analyses are shown in table 2. Dehydroascorbic acid is unstable in milk.

TABLE 2

Reduced ascorbic acid and dehydroascorbic acid during storage of commercial raw milk samples (values in mg. per liter)

Time in days after milking							
1		2		4		6	
Reduced ascorbic acid	Dehydro-ascorbic acid	Reduced ascorbic acid	Dehydro-ascorbic acid	Reduced ascorbic acid	Dehydro-ascorbic acid	Reduced ascorbic acid	Dehydro-ascorbic acid
20.2	3.9	18.8	2.4	15.1	3.7	12.4	2.7
22.8	4.0	19.8	3.2	16.4	4.1	13.4	2.4
18.5	4.7	15.3	4.5	11.3	4.5	7.1	3.3
17.2	6.2	13.2	4.9	7.9	5.8	2.7	4.0
20.5	5.9	15.0	6.2	7.9	8.3	1.3	5.8
19.8	3.3	17.4	2.4	12.7	5.5	8.7	4.4
18.2	5.2	13.6	5.5	7.5	6.2	3.0	4.7
18.2	3.0	15.0	3.4	10.6	5.5	6.7	4.4
14.8	5.4	10.4	5.3	4.8	7.2	0.3	4.7
16.2	4.3	12.9	4.1	7.5	6.2	4.0	4.1
22.1	3.3	19.5	2.8	16.8	3.8	15.4	2.7
19.5	4.3	16.0	4.5	12.7	5.1	10.0	3.1
Ave. 19.0	4.5	15.6	4.1	10.9	5.5	7.1	3.9

The amount present in milk at any time depends on the rate of formation and the rate of destruction of dehydroascorbic acid. As the ascorbic acid disappears from milk the rate of replenishment of the dehydroascorbic acid becomes slower. Table 3 shows the rate of disappearance of dehydroascorbic

TABLE 3

Stability of added dehydroascorbic acid in milk stored at 1° C.

Time, in days, after milking	Start	2	4	6
Mg. dehydroascorbic acid per liter	74.2	34.2	18.3	9.0

acid when added to milk and stored at 1° C. Since dehydroascorbic acid is known to be unaffected by copper or light and sensitive to pH above 4.0 (1, 2, 6), it can be assumed that the destruction of the dehydroascorbic acid

is not due to oxidation but to the effect of pH, presumably by opening of the lactone ring to form 2,3-diketo-l-gulonic acid (5). (Figure 2 shows that oxygen and oxygen plus copper have little, if any, effect on the disappearance of dehydroascorbic acid from milk during storage.)

The examples in table 2 are commercial milk to which no copper was added. If copper is added to the milk, the amount of dehydroascorbic acid which accumulates is increased. Figure 1 shows the curves for the drop in reduced ascorbic acid and total vitamin C in milk containing 0.1 mg. of added copper per liter. For comparison, the average values from table 2 are also plotted, showing the corresponding decreases in milk to which no copper has been added. It can be seen that on the second day at 1° C., for example, 71 per cent of the vitamin C in the milk contaminated with copper

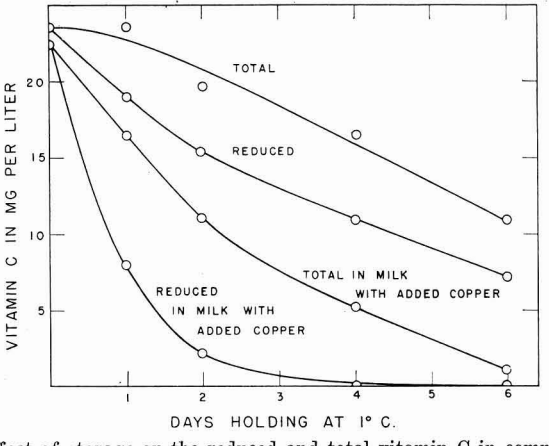

FIG. 1. Effect of storage on the reduced and total vitamin C in commercial milk.

is in the form of dehydroascorbic acid. In the milk to which no copper was added the percentage of vitamin C in the form of dehydroascorbic acid was about 25 on the second day. Commercial milk, arriving at New York City in tank cars and trucks representing 3500 cans averaged 17.4 mgm. per liter of total ascorbic acid of which 4.2 mgm. or 24 per cent was in the dehydro form (10).

The data described so far apply to raw milk. Pasteurization destroys the dehydroascorbic acid which has accumulated in the milk at the time of heating. There is at the same time a slight loss of reduced ascorbic acid during pasteurization. Table 4 shows the results of experiments made on 12 samples of mixed commercial milk taken from cans at the time of delivery and held at 10° C. for 24 hours before pasteurization. Each sample was divided so as to provide a raw control. Pasteurization was carried out in half-filled quart bottles, for 30 minutes at 63° C., with protection from light. The pasteurized and raw samples were titrated simultaneously and

TABLE 4

Effect of pasteurizing on the ascorbic acid and dehydroascorbic acid in milk (values in mg per liter)

Raw			Pasteurized			Loss during pasteurization		
Reduced ascorbic acid	Dehydro-ascorbic acid	Total vitamin C	Reduced ascorbic acid	Dehydro-ascorbic acid	Total vitamin C	Reduced ascorbic acid	Dehydro-ascorbic acid	Total vitamin C
20.5	2.1	22.6	17.2	1.2	18.4	3.3	0.9	4.2
19.9	1.8	21.7	16.6	0.9	17.5	3.3	0.9	4.2
19.6	3.6	23.2	16.3	0.6	16.9	3.3	3.0	6.3
17.8	3.6	21.4	14.3	1.2	15.5	3.5	2.4	5.9
16.9	3.6	20.5	14.0	0.9	14.9	2.9	2.7	5.6
17.2	1.8	19.0	15.5	0.5	16.0	1.7	1.3	3.0
19.0	1.8	20.8	16.6	0.6	17.2	2.4	1.2	3.6
19.0	1.8	20.8	16.3	0.9	17.2	2.7	0.9	3.6
18.4	2.7	21.1	15.7	0.9	16.6	2.7	1.8	4.5
19.3	1.8	21.1	17.2	0.6	17.8	2.1	1.2	3.3
17.5	2.7	20.2	14.8	0.7	15.5	2.7	2.0	4.7
13.7	3.5	17.2	10.1	0.9	11.0	3.6	2.6	6.2
Ave. 18.2	2.6	20.8	15.4	0.8	16.2	2.8	1.8	4.6

to the same endpoint, so that the observed differences would be entirely reliable. The data in table 4 show that the raw milk contained 2.6 and the pasteurized milk 0.8 mgm. of dehydroascorbic acid per liter or a decrease of 1.8 mgm. At the same time the reduced ascorbic acid decreased 2.8 mgm. per liter or more than enough to replace all of the dehydroascorbic acid present in the milk at the beginning of pasteurization. The total loss was 4.6 mgm. with only 0.8 present as dehydroascorbic acid at the end of the treatment. This indicates that all of the dehydroascorbic acid present at the beginning of pasteurization and some of the dehydroascorbic acid formed during the pasteurization was converted to the inactive form of vitamin C.

Commercial pasteurization and bottling has two additional incidental effects which influence the relative amounts of ascorbic and dehydroascorbic acids in the milk: introduction of copper and exposure to light, both of which in the presence of oxygen cause a loss of reduced ascorbic acid and the accumulation of dehydroascorbic acid. The effect of the copper continues during subsequent holding. This explains the wide variations previously encountered (4, 9, 10, 11, 12, 13).

TABLE 5

Effect of temperature on stability of dehydroascorbic acid in milk. (Whole milk, pasteurized, containing added dehydroascorbic acid)

Temperature	Mg. dehydroascorbic acid per liter			
	Start	1 hr.	2 hrs.	3 hrs.
0°	83.4	83.4	82.8	83.4
20°	83.4	74.5	62.0	53.1
40°	83.4	21.7	11.4	7.6

The effect of temperature on the rate of destruction of dehydroascorbic acid is indicated further in table 5. This table indicates that the dehydroascorbic acid present at the beginning of pasteurization would be destroyed by the end of the heating period and that any dehydroascorbic acid found in pasteurized milk would arise only from reduced ascorbic acid oxidized toward the end of pasteurization or subsequent to the heating.

In milk which is deaerated immediately after pasteurizing or is deaerated and then pasteurized, all of the vitamin C is in the form of reduced ascorbic acid. Figure 2 shows the results of experiments on deaerated and deaerated-

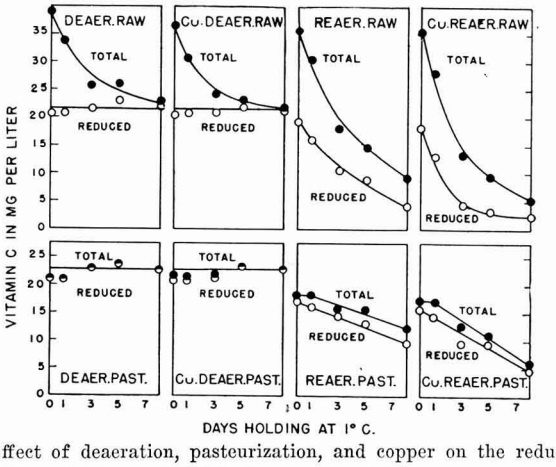

FIG. 2. Effect of deaeration, pasteurization, and copper on the reduced and total vitamin C in milk containing added dehydroascorbic acid.

reaerated milk to which dehydroascorbic acid was added. As can be seen, the dehydroascorbic acid was completely destroyed by pasteurization for 30 minutes and substantially destroyed by storage regardless of the presence or absence of oxygen.

Theoretically there are four stages in the processing of milk at which simple direct titration of reduced ascorbic acid yields an accurate measure of the vitamin C content. They are: directly after milking, directly after pasteurizing, in pasteurized deaerated milk, and several days after complete loss of ascorbic acid; otherwise, simple direct titration of milk may be in error as a measure of total vitamin C content by amounts ranging from 0 to 6 mg. per liter. Except during the heating, the loss of total vitamin C lags behind the loss of reduced ascorbic acid.

SUMMARY

1. Immediately after milking there is no dehydroascorbic acid in cows' milk.

2. During storage the dehydroascorbic acid increases until it supplies an

important fraction of the total vitamin C in the milk. Between 1 and 6 mg. dehydroascorbic acid per liter are generally present in commercial mixed milk provided the milk is not exposed to light or contaminated with appreciable amounts of copper.

3. In milk contaminated with an appreciable amount of copper much of the vitamin C may be present in the form of dehydroascorbic acid as early as 1 day after milking.

4. Pasteurization destroys most of the dehydroascorbic acid. However, in commercially pasteurized milk, by the time it is bottled and ready for delivery, additional amounts of dehydroascorbic acid are formed.

5. In pasteurized deaerated milk the vitamin C is entirely in the form of reduced ascorbic acid and is stable.

REFERENCES

(1) BALL, ERIC G. Studies on Oxidation-Reduction. XXIII. Ascorbic Acid. Jour. Biol. Chem., **118**: 219–239. 1937.

(2) BORSOOK, H., DAVENPORT, H. W., JEFFREYS, C. E. P., AND WARNER, R. C. The Oxidation of Ascorbic Acid and Its Reduction in Vitro and in Vivo. Jour. Biol. Chem., **117**: 237–279. 1937.

(3) GUNSALUS, I. C., AND HAND, D. B. The use of Bacteria in the Chemical Determination of Total Vitamin C. Jour. Biol. Chem., **141**: 853–858. 1941.

(4) GUTHRIE, E. S., HAND, D. B., AND SHARP, P. F. Oxygen in Milk and its Relation to Destruction of Vitamin C and the Development of the Oxidized Flavor. 12th Ann. Rpt. N. Y. State Assoc. Dairy and Milk Insp. 101–110. 1938.

(5) HERBERT, R. W., HIRST, E. L., PERCIVAL, E. G. V., REYNOLDS, R. J. W., AND SMITH, F. The Constitution of Ascorbic Acid. Jour. Chem. Soc. (Eng.) 1270–1290. 1933.

(6) KON, S. K., AND WATSON, M. B. The Effect of Light on the Vitamin C of Milk. Biochem. Jour., **30**: 2273–2290. 1936.

(7) KON, S. K., AND WATSON, M. B. The Vitamin C Content of Cows' Milk. Biochem. Jour., **31**: 223–226. 1937.

(8) SHARP, P. F. Rapid Method for the Quantitative Determination of Reduced Ascorbic Acid in Milk. JOUR. DAIRY SCI., 21: 85–88. 1938.

(9) SHARP, P. F., GUTHRIE, E. S., AND HAND, D. B. A New Method of Retarding Oxidized Flavor and Preserving Vitamin C—Deaeration. Internatl. Assoc. Milk Dealers, 33rd yr., Bul. 20: 523–545. 1941.

(10) SHARP, P. F., HAND, D. B., AND GUTHRIE, E. S. Experimental Work on Deaeration of Milk. Internatl. Assoc. of. Milk Dealers, 34th yr., Bul. No. 17: 365–375. 1942.

(11) WOESSNER, W. W., ELVEHJEM, C. A., AND SCHUETTE, H. A. The determination of Ascorbic Acid in Commercial Milks. Jour. Nutr., **18**: 619–626. 1939.

(12) WOESSNER, W. W., ELVEHJEM, C. A., AND SCHUETTE, H. The determination of Ascorbic Acid in Evaporated Milk, Powdered Milk and Powdered Milk Products. Jour. Nutr. **20**: 327–338. 1940.

(13) WOESSNER, W. W., WECKEL, K. G., AND SCHUETTE, H. A. The Effect of Commercial Practices on Ascorbic Acid and Dehydroascorbic Acid (Vitamin C) in Milk. JOUR. DAIRY SCI., 23: 1131–1141. 1940.

A COMPARISON OF ACETIC ACID, FED IN THE FORM OF TRIACETIN, WITH GLUCOSE AS A NUTRIENT IN FEEDS*

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Some of the organic acids of lower molecular weight play an important role in the nutrition of ruminants. Recent studies *in vitro* by Woodman and Evans (15) indicated that lactic and pyruvic acids and the volatile fatty acids, especially acetic acid, are the products of cellulose digestion in the rumen of cattle. Forage crops preserved by acid fermentation, such as corn, grass, and legume silages, are becoming an increasingly important part of cattle rations. These feeds contain appreciable quantities of lactic and acetic acids. It is, therefore, of practical and theoretical value to know how efficiently the energy of these low-carbon organic acids is utilized by animals.

LITERATURE REVIEW

Although work has been done on the physiological effects of acetic acid ingestion, no studies of its nutritive value have been reported in the literature. The work reported indicates that acetic acid is not a glucose former (1, 11, 12, 13) but that it is rapidly absorbed and oxidized in the animal system (9, 14).

Lusk (9) fed three grams of acetic acid to a fasting dog and noted a distinct rise in its rate of metabolism from 17.61 to 20.74 large Calories per hour, during the two-hour period following acid ingestion. The respiratory quotient dropped slightly from 0.80 to 0.78 after acid ingestion. The author asserted that ". . . the administration of acetic acid does not reduce the carbon dioxide-combining power of the blood, hence one may conclude that it is rapidly absorbed and oxidized." The theoretical R.Q. of acetic acid, however, is 1.00, and the author could not explain the drop in the R.Q. after acid ingestion.

Loeb (8) reported that acetic acid added to the blood circulating through the liver disappears, whereas the content of acetoacetic acid in the blood leaving the liver increases. Friedmann and Turk (6) found that acetoacetic acid was formed when a solution of acetic acid was perfused through surviving livers that were nearly free of glycogen. MacKay and associates (10) showed that the ingestion of acetic acid by a fasting animal causes a rise in the ketone bodies excreted in the urine.

Lamb and Evvard (7) added acetic acid to the ration of pigs and could find no harmful effect on the animals.

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METHODS

In view of the extreme difficulties encountered in an attempt to determine the net energy value of a single food (see the work of Forbes and his co-workers (5)), an alternate method was chosen for evaluating acetic acid as an energy food. An experiment was set up to compare the nutritive value of a ration containing acetic acid with a ration containing glucose. The rations were fed to albino rats by the paired feeding method, and measurements were made of the growth rates of the animals, the percentage of metabolizable energy of the ration, and the rate of metabolism of the animals receiving the acetic-acid-containing ration compared with that of the animals receiving the glucose-containing ration.

The first step in the experiment was to find a satisfactory means of feeding relatively large amounts of acetic acid to albino rats. It has been shown by Deuel and Milhorat (1) and MacKay *et al.* (10) that large doses of acetic acid or of sodium acetate may produce a decidedly abnormal condition in the digestion system of experimental animals. Flury and Wirth (3) have stated that ethyl acetate has toxic properties. In a preliminary experiment it was found that triacetin (glycerol triacetate) is a compound of acetic acid suitable for feeding. A ration containing 20 per cent triacetin by weight was found to be palatable and it seemed to have no harmful effect on the animal. As a consequence, acetic acid was fed as triacetin throughout the experiment.

Three rations, a basal ration containing glucose, a control ration containing no glucose, and an experimental ration containing triacetin, were made up as follows:

COMPOSITION OF RATIONS

	Basal	*No. 1*	*No. 2*
Casein	18.00 grams	18.00 grams	18.00 grams
Yeast	8.00 "	8.00 "	8.00 "
Soybean oil[1]	4.00 "	4.00 "	4.00 "
Glycerol	8.18 "	8.18 "	.....
Glucose	15.00 "	.....	.....
Triacetin[2]	.....	.....	19.48 "
Starch	40.82 "	40.82 "	40.82 "
O. M. Salts[3]	4.00 "	4.00 "	4.00 "
Cod liver oil	2.00 "	2.00 "	2.00 "
	100.00 "	85.00 "	96.30 "

It will be noted that the three rations differed only in their content of glucose and acetic acid. The hydrolysis of 19.48 grams of triacetin requires 4.82 grams of water and yields 8.22[4] grams of glycerol and 16.08 grams of

[1] Corn oil used during latter part of experiment.

[2] Eastman's practical grade triacetin.

[3] Osborne-Mendel salt mixture (see J. Biol. Chem., 15: 317, 1913).

[4] Through error, 8.18 grams of glycerol were used in place of 8.22 grams. The difference is negligible.

acetic acid. In caloric value 16.08 grams of acetic acid are equivalent to 15.00 grams of glucose. Therefore, 100 grams of the basal ration and 96.3 grams of ration No. 2 were equivalent in nutrients and in gross energy, whereas 85.00 grams of ration No. 1 were equivalent in protein and other nutrients but contained less gross energy by an amount equal to 15.00 grams of glucose or 16.08 grams of acetic acid. The rations were fed in equivalent amounts, that is, 100 grams of the basal ration were equivalent to 85 grams of ration No. 1 and to 96.3 grams of ration No. 2.

GROWTH

Four pairs of rats (three pairs of males and one pair of females) were given the basal ration and ration No. 1 for a period of 51 days. The mean growth curves of the three pairs of males are shown in figure 1 and the growth curves of the pair of females are shown in figure 2. The growth rates of the rats given the basal ration were greater in all cases, indicating that the difference in energy intake imposed on the paired animals was great enough to affect their growth curves.

Twenty pairs of rats were given the basal ration and ration No. 2. Their mean growth curves are given in figures 3 and 4; figure 3 represents the data on eleven pairs of males and figure 4 on nine pairs of females. The growth rates on these two rations were essentially equal, indicating that the acetic acid of ration No. 2 was utilized as efficiently as was the glucose of the basal ration.

HEAT PRODUCTION

After the rats had passed the period of rapid growth and were approaching maturity, the heat production of the male animals paired on the basal ration and ration No. 2 was measured by the Haldane open-chain method of indirect calorimetry. The apparatus used was similar to that described by Forbes, Kriss, and Miller (4) and the calculations were made according to the procedure described in that article. The apparatus was made to accommodate two animals.

The heat production of both animals of a pair was measured simultaneously. On the morning of the experiment, the two rats in question were given 6.0 grams of the basal ration and an equivalent amount of ration No. 2, respectively. They were allowed from forty minutes to one hour to clean up their feed, and were placed in the respiration apparatus shortly after this was accomplished. A thirty-minute period was allowed for the rats to become accustomed to the respiration chamber (the heat production for this period is not reported) after which the hourly heat production was measured for five or six hours.

It was found that the method was not entirely reliable in measuring the respiratory quotient. The individual R.Q.'s from twenty experiments are

TABLE 1

Respiratory quotients of albino rats on the basal ration and on ration No. 2

Pair No.	Respiratory quotients	
	Basal ration	Ration No. 2
9	0.844	0.898
9	0.973	0.977
10	0.838	1.112
10	1.055	1.141
21	1.253	1.095
21	0.724	0.789
26	1.438	1.017
26	0.899	1.071
26	0.873	1.034
28	1.192	1.124
28	1.100	1.210
29	1.067	0.948
29	0.938	0.931
30	0.767	1.035
30	1.062	0.973
34	1.143	0.857
34	0.931	0.819
35	1.007	1.070
35	0.822	0.905
36	1.026	0.872
Mean and its probable error	0.9976 ± 0.0197	0.9939 ± 0.0170

given in table 1. In calculating heat production from the respiratory exchange the mean R.Q. of all the experiments was used.

The mean hourly heat production values, expressed as calories per square meter of body surface per hour, are given in table 2. Surface area was calculated from the formula of Diak (2), according to which the surface area in square centimeters equals 7.47 times the two-thirds power of the weight in grams.

A statistical analysis of the data showed that there was no significant

TABLE 2

Mean hourly heat production of albino rats on the basal ration and ration No. 2

Time after feeding in hrs.	Heat production*	
	Basal ration	Ration No. 2
2	39.06 ± 0.51†	38.18 ± 0.71†
3	35.77 ± 0.40	36.08 ± 0.54
4	33.98 ± 0.64	35.09 ± 0.60
5	32.38 ± 0.69	34.32 ± 0.65
6	32.63 ± 0.64	33.11 ± 0.62
7	30.10 ± 0.52	30.05 ± 0.51

* Small calories per square meter of body surface per hour.
† Probable error.

difference in the rate of metabolism of the animals given the glucose-containing and the acetic-acid-containing ration, as shown by the means reported in table 2.

METABOLIZABLE ENERGY

Urine and feces collections were taken from the male rats given the basal ration and ration No. 2. The collections were made from the pairs as a unit; the two animals of a pair were in the balance cages for the same period and refusal or waste of food by either one of the two animals was cause to discard the collection from both. The feces collection periods were five days in length, during which the animals were kept continuously in the balance cages. Urine collections were made by two procedures. Samples were taken in conjunction with a few of the feces collections. In this procedure it was found exceedingly difficult, however, to make collections which were not contaminated with feed. Additional collections, therefore, were made by feeding the animals in their regular cages and placing them in the balance cages after they had cleaned up their food. Food intake was restricted to what the animals would clean up in three or four hours. A record was kept of the time the animals spent in the balance cages, and energy loss in the urine was calculated to a 24-hour basis.

TABLE 3

Energy losses in feces from rats on the basal ration and on ration No. 2

Pair No.	Calories per day excreted in feces*		Daily food intake gm. base†	Calories excreted in feces per gm. of food* intake	
	Basal ration	Ration No. 2		Basal ration	Ration No. 2
5	1928	1814	11	175.3	164.9
5	1799	1791	11	163.5	162.8
9	1813	1512	11	164.8	137.5
9	1912	1926	13	147.1	148.2
10	2121	1849	13	163.2	142.2
13	2055	2169	13	158.1	166.8
13	2049	2060	13	157.6	158.5
13	2054	2185	13	158.0	168.1
15	2227	2170	13	171.3	166.9
16	2216	2297	13	170.5	176.7
16	2347‡	2258	13	180.5	173.7
18	2353	2009	13	181.0	154.5
18	2341	2447	15	156.1	163.1
22	2322	2343	15	154.8	156.2
22	2173	2317	13	167.2	178.2
22	2084	2162	13	160.3	166.3
23	2033	2091	13	156.4	160.8
Mean and its probable error				163.9 ± 1.5	161.5 ± 1.8

* Small calories.
† Intake of ration No. 2 was equivalent in calories to the intake of the basal ration.
‡ Not determined in duplicate.

The feces samples were air dried and the urine samples were dried to constant weight at 100° C. Aliquots of both were burned in duplicate (unless otherwise noted) in an oxygen bomb calorimeter. The energy losses in the feces and urine are given in tables 3 and 4, respectively.

The determined gross energy content of the basal ration and of ration No. 2 was 4,360 and 4,466 small calories per gram, respectively. From these figures, 100 grams of the basal ration were equivalent to 436 large Calories, and 96.3 grams of ration No. 2 were equivalent to 430 large Calories. This

TABLE 4

Energy losses in urine from rats on the basal ration and ration No. 2

Pair No.	Length of period	Calories per day excreted in urine*		Daily food intake gm. base	Calories excreted in urine per gm.* of food intake	
		Basal ration	Ration No. 2†		Basal ration	Ration No. 2
	hours					
5‡	120.0	821	932	11	74.6	84.7
13‡	120.0	1070	945	13	82.3	72.7
18‡	120.0	963	1154	15	64.3	76.9
22‡	120.0	917	1038	15	61.1	69.2
23‡	120.0	931	937	13	71.6	72.4
10	104.4	895§	838§	10	89.5	83.8
21	103.2	776§	901§	10	77.6	90.1
26	81.0	806§	864§	10	80.6	86.4
26	105.3	982	924	10	93.2	92.4
28	102.6	652§	594§	10	65.2	59.4
29	124.7	899	1035	10	89.9	103.5
34	102.2	672§	797	10	67.2	79.7
34	107.3	713	1025	10	71.3	102.5
Mean and its probable error					74.4 ± 2.3	82.1 ± 2.2

* Small calories.
† Intake of ration No. 2 was equivalent in calories to the intake of the basal ration.
‡ Urine samples taken in conjunction with feces collections.
§ Not determined in duplicate.

experimentally justified the caloric equivalent of 100 grams of base to 96.3 grams of ration No. 2, the ratio in which the rations were fed. When the loss of energy in the urine and feces per gram of food intake was subtracted from these figures, the metabolizable energy of the basal ration was 4,122 small calories per gram, or 94.5 per cent of the gross energy, and that of ration No. 2 was 4,211 small calories per gram, or 94.3 per cent of the gross energy.

Volatile acids were determined on aliquots of several of the urine collections, by a modification of the method described by Deuel and Milhorat (1). The results, expressed as milligrams of acetic acid excreted per gram of food intake, are given in table 5.

TABLE 5

Loss of volatile acids in urine from rats on the basal ration and on ration No. 2

Pair No.	Mgm. acetic acid per gram of food intake	
	Basal ration	Ration No. 2
10	9.20	11.12
10	3.89	4.08
21	4.91	4.98
21	5.36	5.37
26	5.34	6.00
28	4.68	3.34
29	5.15	4.34
30	5.75	4.71
30	3.94	4.16
34	4.88	4.92
34	3.25	3.72
35	5.00	5.00
Mean and its probable error	5.11 ± 0.28	4.60 ± 0.15

DISCUSSION

One of the questions that must be answered in an experiment of this type is whether or not the experimental substance was fed in quantities which were large enough so that the lack of variation in the value of the two rations may be interpreted as significant. The growth study comparing the basal ration and ration No. 1 (figures 1 and 2) was made for this purpose. In effect, ration No. 1 was the same as ration No. 2 if the acetic acid contained

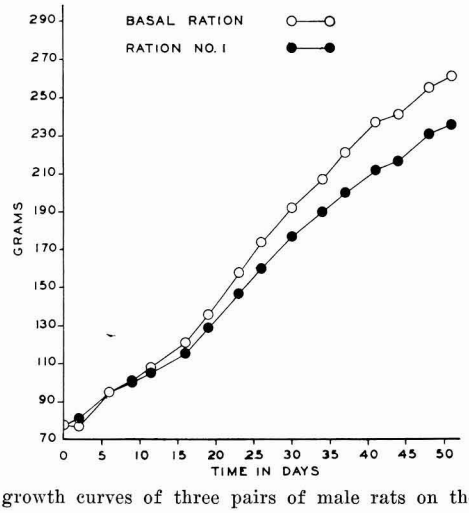

FIG. 1. Mean growth curves of three pairs of male rats on the basal ration and ration No. 1.

in ration No. 2 was of no value to the animals. The comparison of the basal ration with ration No. 1 is based on only four pairs of animals, but the differences in growth rates are large enough and consistent enough to leave little doubt that if the acetic acid contained in ration No. 2 had not been utilized, the growth curves of the animals on this ration would have fallen below those of the rats given the basal ration. The growth curves shown in figures 3 and 4 are interpreted as indicating that the acetic acid contained in ration No. 2 was utilized by the albino rats.

The balance studies show that the acetic acid of ration No. 2 was not excreted in the urine of the animals and that the gross energy of this ration was retained by the animals as efficiently as was that of the basal ration.

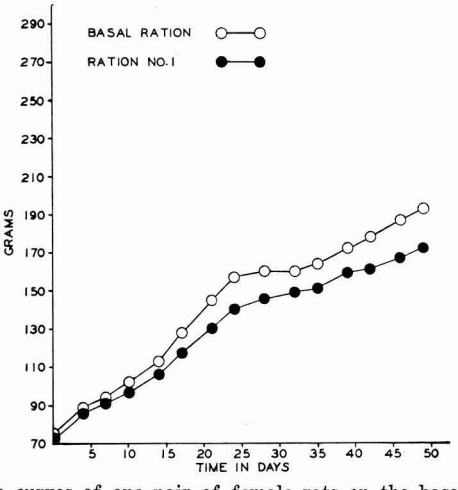

FIG. 2. Growth curves of one pair of female rats on the basal ration and ration No. 1.

The heat production measurements show that there was no measurable difference between the two rations in their effect on the rate of metabolism of the animals. The studies taken together show that, within the limits of accuracy of the experiment, the two rations were equal.

The fact that ration No. 2 caused no pronounced increase in the rate of metabolism appears contrary to the common belief that acetic acid has a high specific dynamic effect. Lusk (9) found that three grams of acetic acid (fed as a dilute solution) caused the heat production of a fasting dog to increase to 125 per cent of the basal level. As compared to this, the rats receiving ration No. 2 were given 0.96 gram of acetic acid, in the form of triacetin, prior to the heat production measurements.

In proportion to the weights of the animals this is several times as much acetic acid as Lusk was able to feed the dog and it caused no greater increase in heat production than a like ration containing glucose.

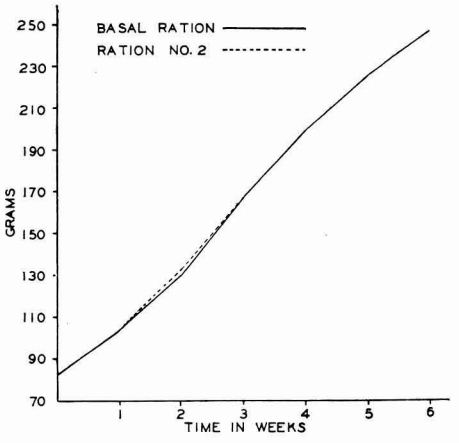

FIG. 3. Mean growth curves of eleven pairs of male rats on the basal ration and ration No. 2.

It is believed that this difference in results is due to the difference in the forms of acetic acid fed. As McKay *et al.* (10) have said that acetic acid, even when fed by stomach tube, acts as an emetic, it is possible that the high specific dynamic effect found by Lusk was, in reality, an increase in metabolism resulting from the shock effect of the acid on the nervous system of the dog.

The fact that acetic acid does not have a high specific dynamic effect is in harmony with the theory that it is the product of beta-oxidation of fat; the specific dynamic effect of fats is known to be low. Work has been cited (6, 8, 10) which shows that acetic acid may be converted to acetone bodies in a fasting animal. MacKay *et al.* (10) presented convincing evidence in support of this theory and they postulated that, in the fat metabolism of a

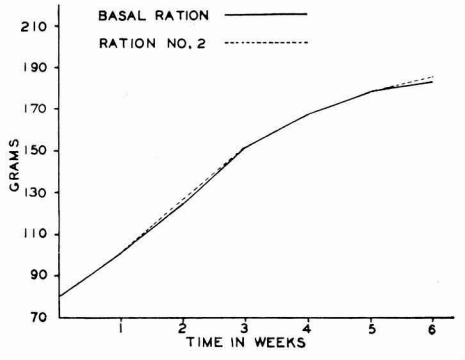

FIG. 4. Mean growth curves of nine pairs of female rats on the basal ration and ration No. 2.

normal animal, acetic acid produced by beta-oxidation is either oxidized directly or converted to acetone bodies that are oxidized.

SUMMARY AND CONCLUSIONS

A study of the nutritive value of two balanced rations has been conducted. The first of these rations, called the basal ration, contained 15 per cent glucose. The second ration, designated as ration No. 2, contained acetic acid in the form of triacetin equal in caloric value to the glucose of the basal ration. They were alike in all other respects. The two rations were fed to albino rats, and the food intake was controlled by the method of paired feeding.

A comparison of the efficiency of the rations in supporting growth of immature rats showed that the two rations were equivalent in this respect.

No difference in the rate of metabolism of the rats on the two rations could be detected.

A study of the metabolizable energy of the two rations revealed that the energy of the basal ration was 94.5 per cent metabolizable and that of ration No. 2, in which acetic acid as triacetin was substituted for an equivalent amount of glucose, was 94.3 per cent metabolizable. The difference between the two rations was not significant.

There was no significant difference in the excretion of volatile acids in the urine of rats on the two rations.

Acetic acid, in the form of triacetin, fed over a 7-month period had no harmful effect on the animals.

The basal ration and ration No. 2 were, therefore, exactly equivalent in value when used to support growth and for maintenance of rats. From this, it is concluded that acetic acid, in the form of triacetin, was utilized with a degree of efficiency equal to that of glucose.

REFERENCES

(1) DEUEL H. J., JR., AND MILHORAT, A. T. The Alleged Conversion of Fat to Carbohydrate. I. The Metabolism of Acetic Acid. Jour. Biol. Chem., 78: 299. 1928.

(2) DIAK, S. L. Determination of the Surface Area of the White rat. Jour. Nutr. 3: 289. 1930.

(3) FLURY, F., AND WIRTH, W. The Toxicity of Organic Solvents (Various Esters, Acetone and Methanol). Arch. Gewerbepath. Gewerbehyg., 5: 1, 1933. Cited from Chem. Abs., 28: 1776. 1934.

(4) FORBES, E. B., KRISS, M., AND MILLER, R. C. The Energy Metabolism of the Albino Rat in Relation to the Plane of Nutrition. Jour. Nutr. 8: 535. 1934.

(5) FORBES, E. B., AND SWIFT, R. W. The Minimum Base Value of Heat Production in Animals. A Research in the Energy Metabolism of Cattle. Pa. Agr. Expt. Sta. Bul., 415. 1941.

(6) FRIEDMANN, E., AND TURK, W. Decomposition of the Carboxylic Acids in the Animal Body. XVII. Formation of Acetoacetic Acid from Acetic Acid in Perfusion of the Liver. Biochem. Z., 55: 436. 1913, from Chem. Abs., 8: 1169. 1914.

(7) LAMB, A. R., AND EVVARD, J. M. The Acid Base Balance in Animal Nutrition. I. The Effect of Certain Organic and Mineral Acids on the Growth, Well Being and Reproduction of Swine. Jour. Biol. Chem., 37: 317. 1919.

(8) LOEB. A. Behavior of Acetic Acid During Artificial Circulation of Blood through the Liver. Biochem. Z., 47: 118, 1912. Cited from Chem. Abs., 7: 1901. 1913.

(9) LUSK, G. The Behavior of Various Intermediary Metabolites Upon the Heat Production. Jour. Biol. Chem., 49: 453. 1921.

(10) MACKAY, E. M. BARNES, R. H., CARNE, H. H., AND WICK, A. M. Ketogenic Activity of Acetic Acid. Jour. Biol. Chem., 135: 157. 1940.

(11) MAJER. E. H., AND REISNER, H. Influence of Different Organic Acids on the Carbohydrate-Accumulating Capacity of the Rat Liver. Biochem. Z., 263: 340, 1933. Cited from Chem. Abs., 27: 583. 1933.

(12) PONSFORD, A. P., AND SMEDLY-MACLEAN. The Influence of Succinic, Fumaric, Malic and Acetic Acids on the Deposition of Liver Glycogen. Biochem. Jour., 26: 1340. 1932.

(13) RINGER, A. I., AND LUSK, G. The Origin of Dextrose from Amino Acids in Phlorhizin Glucosuria. Z. Physiol. Chem., 66: 106. 1910. Cited from Chem. Abs., 5: 709. 1911.

(14) TAISTRA, S. A. The Influence of Acids upon the Carbon Dioxide-Combining Power of the Blood Plasma. Jour. Biol. Chem., 49: 479. 1921.

(15) WOODMAN, H. E., AND EVANS, R. E. The Mechanism of Cellulose Digestion in the Ruminant Organism. Jour. Agr. Sci., 28: 43. 1938.

THE PREVENTION OF OXIDIZED FLAVOR IN MILK AND ICE CREAM BY THE USE OF CONCENTRATED MILK PRODUCTS[1]

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Certain constituents of milk have been shown to be antioxidants. Kende (10) pointed out in 1934 that the ascorbic acid in milk was part of the reducing system that protected against oxidation; however, he believed that ascorbic acid was only a part of a complex reducing system. Gould (7) reported that substances other than ascorbic acid constituted from 40 to 60 per cent of the reducing system. Corbett and Tracy (2, 3) have observed that certain amino acids, particularly tyrosine and its ethyl ester, may be natural antioxidants. Barnicoat and Palmer (1) reported that the soluble phosphates and citrates appeared to account for much of the antioxigenic effect of milk plasma. Gould and Sommer (8) and Josephson and Doan (9) have shown that a reducing system produced when milk is heated to high temperatures is effective in retarding oxidized flavor. The process of milk condensing was demonstrated by Corbett and Tracy (2) to retard the oxidized flavor.

Dry milk may also be an antioxidant. As early as 1931, Dahle *et al.* (6) found that ice cream containing dry skimmilk did not develop "a peculiar cardboard or tallowy flavor" noticed in the control samples. When Dahle and Folkers (5) found less copper in ice cream made with dry milk, they reasoned that this accounted for its superior storage qualities. However, Dahle (4) pointed out later that the copper content was not the only factor involved, but that dry milk itself may have antioxygenic properties.

Incidental to some experiments on homogenization, Trout and Gould (14) observed that when dry skimmilk was added to milk, oxidized flavor decreased in intensity or was prevented entirely, partially due perhaps to the flavor imparted by the higher concentrations of the dry skimmilk powder. Musher (11) was granted a patent in 1940 on the addition of concentrated or dried milk-solids-not-fat to cream followed by a heat treatment to stabilize the flavor of the cream and products made from it. Corbett and Tracy (3) observed that a slight antioxidant effect was given by skimmilk powder.

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EXPERIMENTAL

These experiments were planned to compare the antioxigenic ability of various types of concentrated milk. Every attempt was made to eliminate all variables other than the one being studied. Except for the commercial skimmilk powder used in a preliminary experiment, all concentrated milks were manufactured in the College Creamery, using copper-free equipment, each series being prepared from a single lot of milk. The lot of milk was first concentrated as described in each experiment in a stainless-steel, 16-inch vacuum pan, and was cooled immediately over a small newly tinned surface cooler. Portions of the condensed milk were dried on a 6-inch double roll Buflovak drier which could be operated either under atmospheric pressure or under a 28-inch vacuum. Both the condensed milk and powdered milk samples were stored in glass containers at 32° F. Care was taken to keep the bacterial content, agitation and handling at a minimum and to exclude light as much as possible. The concentrated milks were added to fresh milk as described in the experiments and the milk was judged for oxidized flavor each day by three judges. The samples were scored individually, using the following rating: 0 = no oxidized flavor, 0.5 = questionable oxidized flavor, 1 = definite oxidized flavor, 2 = pronounced oxidized flavor, 3 = very pronounced oxidized flavor, and 4 = extremely pronounced oxidized flavor.

Ice cream samples were prepared according to a procedure which was found during preliminary studies to produce comparable results with the ice cream processed in the usual manner, and yet was economical in time and milk products. Maximum amounts of the concentrates studied were included in the mix to accentuate if possible the effect of the variable. Under this method the mixes were composed of concentrated milk, distilled water, cream, sugar and gelatin, and the composition of the mix was as follows: 13 per cent fat, 10.5 per cent serum solids, 15.5 per cent sugar, and 0.3 per cent gelatin. The only variable in the mixes were the type and amount of concentrated milk and the amount of distilled water added. However, in a series in which concentrated skimmilk and concentrated whole milk were compared, more cream necessarily was added to the mixes containing concentrated skimmilk.

The mixes were pasteurized in glass at 150° F. for 30 minutes, then cooled to approximately 34° F., and held overnight at that temperature. The following day 3 p.p.m. copper were added to all samples except the control immediately before freezing in a $\frac{1}{2}$ gallon vertical freezer. The mixes were not homogenized in order that the flavor defect would appear more quickly in the control samples. The time of freezing and whipping was determined on the control batch and an interval timer regulated the following batches. The ice cream was removed from the freezer, packed in pint cartons, and stored in the hardening room. On the day before the samples were scored, a pint of each sample in the series was tempered in a

10° F. cabinet; a fresh set of samples being scored each period. The judges and the method of scoring were the same as for the milk.

RESULTS

The Antioxidant Effect of Commercial Dry Skimmilk in Milk

From the literature it was evident that dry skimmilk would no doubt aid in retarding oxidized flavor in ice cream. As a preliminary experiment it was decided to test the effect of commercial skimmilk powder on oxidized flavor of milk. Dry skimmilk of two types, a "high-heat" powder and a "low-heat"[3] powder, was added to milk in amounts equal to 0.2, 0.4 and 0.6 per cent of the total weight. The fluid milk in this case was pasteurized milk from the College market milk department. The bottles were shaken until no visible particles of dry milk remained. Copper was added from a solution of copper sulphate to part of the samples so that they contained one p.p.m. of added copper.

Table 1 indicates that the dry skimmilk prevented oxidized flavor in

TABLE 1

Preliminary trial showing the effect of small additions of dry skimmilk on oxidized flavor development in pasteurized milk*

Amount and type of dry skimmilk added to pasteurized milk	Degree of oxidized flavor after storage							
	No Cu added				1 p.p.m. Cu added			
	2 days	3 days	4 days	5 days	2 days	3 days	4 days	5 days
Pasteurized milk control	0.0	1.0	2.0	2.0	0.0	2.0	4.0	4.0
" " +0.2% "low-heat" powder	0.0	0.5	2.0	2.0	0.0	1.0	1.5	2.0
" " +0.4% " " "	0.0	0.0	1.0	1.0	0.0	1.5	1.5	2.0
" " +0.6% " " "	0.0	0.0	0.0	0.0	0.0	0.5	1.0	1.0
" " +0.2% "high-heat" "	0.0	2.0	2.0	2.0	0.0	3.0	3.0	4.0
" " +0.4% " " "	0.0	1.0	1.0	1.0	0.0	2.5	2.0	2.0
" " +0.6% " " "	0.0	0.5	1.0	1.5	0.0	1.5	2.0	3.0

* Commercial dry skimmilk was about six months old at the time used.

some instances and decreased the intensity of the flavor in nearly all samples. In this respect the "low-heat" powder was at least equal to and usually superior to the "high-heat" powder. These results were such as to encourage further investigation, especially since the history of the powder was unknown other than it was at least six months old, and the methods used in preparation of the samples were relatively unknown, except that in one case the milk was processed while in the fluid state at a high temperature, while in the other case a lower temperature was used.

The control sample of pasteurized milk developed oxidized flavor without any addition of copper. It is evident that the solids of dry skimmilk

[3] Designation by manufacturer.

have antioxigenic properties, although the product heated to the highest temperature did not prove superior as was expected.

Comparison of Various Heat-Treatments of the Condensed Skimmilks in Retarding Oxidized Flavor

From the literature it is apparent that both the condensing process and the heating of milk to high temperatures retard oxidized flavor. It was reasoned that condensed skimmilk added to milk might retard oxidized flavor development and that the heat treatments which the skimmilk received, such as in preheating before condensing or superheating after condensing, might enhance its qualities as an antioxidant.

Three kinds of condensed milk were prepared from a batch of raw skimmilk as follows: 1) Low temperature condensed milk. This was concentrated at a 25-inch vacuum so that the highest temperature attained was 135° F., 2) Preheated condensed skimmilk. The skimmilk was forewarmed to 205° F. and then concentrated, and 3) Superheated condensed skimmilk. This was made by superheating a part of the "low temperature condensed milk" to 190° F. These will later be referred to as low temperature, preheated and superheated condensed skimmilk.

Samples of the three types of condensed skimmilks were added to pasteurized fluid milk at the rate of 0.2 per cent milk solids-not-fat. In table 2 may be seen the results of three trials. It can be observed that even the low temperature condensed skimmilk decreased oxidized flavor when added at the rate of only 0.2 per cent milk solids-not-fat. However, the antioxidant effect was more noticeable with the highly heated condensed skimmilk. No cooked flavor was evident in any of these samples.

TABLE 2

Comparison of the effect on oxidized flavor development when 0.2 per cent M.S.N.F. of differently heated condensed skimmilks were added to milk after pasteurization. Average of three trials

Type of condensed skimmilk added at rate of 0.2% M.S.N.F. to pasteurized milk	Oxidized flavor after storage		
	1 day	2 days	3 days
Control	1.0	1.6	3.0
" +"low temperature" condensed skimmilk	0.5	1.1	2.0
" +"preheated" condensed skimmilk	0.0	0.5	1.5
" +"superheated" condensed skimmilk	0.0	0.3	1.2

Optimum Heat Treatment of Dry Skimmilk

In preliminary trials as shown in table 1, commercial dry skimmilk had some antioxidant properties, the "low-heat" being somewhat superior to the "high-heat." This experiment was repeated using four kinds of dry fresh skimmilk, all prepared from one lot of fluid skimmilk in copper-free equipment. The heat treatment received by the dry skimmilk varied from a low

temperature condensed milk dried under 28 inches of vacuum to a superheated condensed milk dried atmospherically over the rolls carrying 35 pounds of steam pressure. These four dry milks were added in various concentrations to fresh milk after the method given in the preceding experiment. For the sake of brevity, the results obtained from adding 0.2 per cent milk solids-not-fat in the form of dry skimmilk are shown in table 3. These dry skimmilk samples all retarded oxidized flavor, but in this case the dry skimmilk heated to the highest temperatures had the greatest antioxidant effect.

TABLE 3

Comparison of the effect of oxidized flavor development when four types of dry skimmilk were added to milk after pasteurization

Type of dry skimmilk added at rate of 0.2% M.S.N.F. to pint bottles of pasteurized milk	Oxidized flavor after storage					
	No Cu added			1 p.p.m. Cu		
	2 days	3 days	4 days	2 days	3 days	4 days
Control	0.0	1.0	2.0	2.0	3.0	4.0
" + low temperature vacuum drum dry skimmilk	0.0	0.0	2.0	1.0	2.0	4.0
" + " " atmospheric " " "	0.0	0.0	2.0	1.0	1.0	4.0
" + high " vacuum " " "	0.0	0.0	1.0	0.0	0.5	3.0
" + " " atmospheric " " "	0.0	0.0	0.5	0.0	0.5	2.0

Effect of Source of Milk Solids-Not-Fat on Oxidized Flavor of Ice Cream

Three types of condensed skimmilk were prepared from the same lot of fluid skimmilk designated as low temperature, preheated, and superheated condensed skimmilk. Half of each sample of condensed skimmilk was dried atmospherically. Using these six sources of milk-solids-not-fat, ice cream mixes were prepared according to the methods given under experimental procedures. No fluid skimmilk was added to these mixes, but 2.5 per cent

TABLE 4

Comparison of resistances of ice cream toward oxidized flavor development when prepared from various concentrated milks

Mixes contained 2.5 per cent M.S.N.F.* from the following concentrates	Oxidized flavor after storage†				
	7 days	14 days	21 days	28 days	48 days
Low temperature condensed skimmilk	0.0	1.0	1.1	1.3	3.0
Preheated " "	0.0	0.5	1.0	1.1	2.0
Superheated " "	0.0	0.3	0.3	0.0	2.0
Low temeperature dry skimmilk	0.0	0.0	0.3	0.0	1.0
Preheated " "	0.0	0.0	0.0	0.0	1.0
Superheated " "	0.0	0.0	0.0	0.0	1.0

* The rest of the milk solids-not-fat was supplied by low temperature condensed skimmilk and cream.

† 3 p.p.m. copper added before freezing.

of the milk solids-not-fat was supplied by the various concentrates and the rest was made up by adding a uniform amount of low temperature condensed to each mix and varying amounts of distilled water. Two and one-half per cent milk solids-not-fat was used as that is about the average amount of milk solids-not-fat added by the concentrate in commercial mixes when fluid skimmilk is used. The results of this trial appear in table 4. Atmospheric drying of the condensed skimmilk definitely increased the antioxidant properties. The three types of skimmilk powder were about equally effective in retarding oxidized flavor in ice cream, while the condensed skimmilks were in the same relation as was evidenced in milk (table 2).

Comparison of Oxidized Flavor Development in Reconstituted Condensed Whole Milk and Skimmilk

Corbett and Tracy (2) reported that when condensed whole milk and condensed skimmilk were reconstituted to fluid milk, oxidized flavor was retarded. However, they gave no indication as to how they compared in this respect with each other. An experiment was therefore planned to compare the ability of condensed whole milk and condensed skimmilk in retarding oxidized flavor when reconstituted to fluid milk and also when included in ice cream mix.

A batch of whole milk was pasteurized and divided into two parts. One half was used to make the condensed whole milk and the other was separated to provide the condensed skimmilk series. The usual treatments were used in the preparation of the low temperature, preheated and superheated samples.

The samples of condensed whole milk were reconstituted with distilled water to contain 4 per cent butterfat. The samples of condensed skimmilk were reconstituted to test 4 per cent butterfat and 9 per cent milk solids-not-fat with fresh 41.5 per cent cream and distilled water. This cream was not separated from the original batch of whole milk, but came from a similar batch of milk produced two days later. However slight this difference, it does constitute an additional variable between the condensed whole milk and the condensed skimmilk in this series. Three p.p.m. of copper were added to all samples after reconstitution.

Table 5 indicates that none of the reconstituted condensed whole milk samples became oxidized in nine days even with the 3 p.p.m. of copper added, whereas all of the reconstituted condensed skimmilk samples had developed the flavor to some degree.

The same lots of condensed milks were used to make ice cream mixes. In order to accentuate the differences in retarding oxidized flavor due to the treatment of the concentrated milk, all milk solids-not-fat except that supplied by the cream came from the condensed milk studied. While more cream was required in mixes using condensed skimmilk than those using

TABLE 5

Comparison of oxidized flavor development in milk reconstituted from various types of condensed whole milk and skimmilk

Reconstituted milks* made from	Oxidized flavor after storage			
	36 hrs.	48 hrs.	168 hrs.	216 hrs.
Low temperature condensed skimmilk	0.3	0.0	2.0	3.0
Preheated " "	0.3	0.0	2.0	3.0
Superheated " "	0.0	0.0	0.0	1.0
Low temperature " whole milk	0.0	0.0	0.0	0.0
Preheated " " "	0.0	0.0	0.0	0.0
Superheated " " "	0.0	0.0	0.0	0.0

* Three p.p.m. copper added after reconstitution.

condensed whole milk, the difference was not great. Table 6 shows the degree of oxidized flavor development after storage. Again the condensed whole milk was much superior to the condensed skimmilk in retarding oxidized flavor. Both superheated products developed less oxidized flavor than either the low temperature or preheated types, which is further evidence that high heat treatment is beneficial to the antioxidant properties of the condensed milk, especially if such treatment occurs after condensing.

TABLE 6

Effect of various types of condensed milk on development of oxidized flavor in ice cream

Condensed milk supplying maximum amount of M.S.N.F. of mix*	Oxidized flavor† after storage			
	3 days	7 days	10 days	14 days
Low temperature condensed skimmilk	1.0	2.6	4.0	4.0
Preheated " "	1.5	3.0	4.0	4.0
Superheated " "	0.0	0.8	1.2	3.0
Low temperature " whole milk	0.0	0.0	0.0	1.0
Preheated " " "	0.0	0.0	0.0	1.0
Superheated " " "	0.0	0.0	0.0	0.0

* All except M.S.N.F. supplied by cream.
† 3 p.p.m. copper was added before freezing.

Effect of Degree of Concentration of Condensed Whole Milk on Its Antioxidant Properties

A single trial on the influence of the degree of concentration of condensed whole milk verified the findings of Corbett and Tracy (2) on this point. Whole milk of 12.47 per cent T.S. was condensed to 15.36, 23.52, 32.44, 35.01, 41.67, 46.56, and 48.72 per cent total solids respectively. When these samples were reconstituted with distilled water to 12.47 per cent total solids, and 3 p.p.m. of copper added, and then stored for 5 days, the uncondensed sample had an oxidized flavor score of 3.6; the 15.36 per cent a score of 1.1; and the 23.52 per cent and higher concentrations had developed no oxidized flavor.

Comparison of Antioxidant Abilities of Various Kinds of Concentrated Milk When Added to Milk Before Pasteurization

A preliminary trial indicated that if the concentrated milk was added to milk before pasteurization, it was more effective in retarding oxidized flavor development. A full series of concentrated milks were prepared from a single batch of whole milk including low and high temperature condensed skimmilk and low and high temperature condensed whole milk. The four types of condensed milks were dried under vacuum so that eight different concentrated milks were available. Enough of these concentrated milks was added to the fluid milks so that they contributed 0.6 per cent milk solids-not-fat. Samples of milk were pasteurized with constant and uniform agitation by belt-driven glass agitators. Two p.p.m. of copper were added to the flasks after the milk had been pasteurized and cooled. Since the capacity of this equipment was six samples, it was necessary to pasteurize two lots, which explains the second control in table 7. This trial indicates as did other trials that, other things being equal, the antioxidant power of dry milk was greater than condensed milk; high temperature concentrated milk was greater than low temperature concentrated milk; and concentrated whole milk was greater than concentrated skimmilk.

TABLE 7

Effect on oxidized flavor of adding various types of concentrated milk to fluid milk before pasteurization

Type of concentrated milk added to raw milk at rate of 0.6 per cent milk solids-not-fat before pasteurization*	Oxidized flavor after storage		
	24 hrs.	36 hrs.	60 hrs.
Control (first lot)	2.0	2.0	4.0
Low temperature condensed skimmilk	1.0	1.0	2.0
High " " "	0.5	1.6	2.0
Low " " whole milk	0.0	0.3	0.8
High " " " "	0.0	0.3	1.3
Low " dry skimmilk	0.0	1.0	1.3
Control (second lot)	0.0	0.0	2.0
High temperature dry skimmilk	0.0	0.0	2.0
Low " " whole milk	0.0	0.0	0.8
High " " " "	0.0	0.0	0.0

* Two p.p.m. of copper were added after pasteurization.

Comparison of Antioxidant Abilities of Various Kinds of Concentrated Milk When Included in Ice Cream at the Maximum Concentration

The same eight concentrated milks used in table 7 were each used to furnish 8.75 per cent of the 10 per cent milk solids of ice cream mix. These were processed as described previously. Table 8 reveals the same relation as was observed in table 7, with milk. No oxidized flavor was present in any ice cream containing dry milk, although this ice cream contained 3 p.p.m. of copper. When both the low and high temperature dry skimmilks

were added at the high rate of 8.75 per cent, a pronounced "powder" flavor was present in the ice cream. It was interesting that the samples of ice cream containing whole milk powder had no trace of "powder" flavor and in fact were judged at all periods to have the best flavor of the entire series.

TABLE 8

Effect upon oxidized flavor development of ice cream prepared from the maximum amounts of various concentrated milks

Type of concentrated milk used	Oxidized flavor* after storage			
	7 days	11 days	14 days	17 days
Low temperature condensed skimmilk	1.0	1.0	2.0	3.0
High " " "	1.0	1.0	1.0	3.0
Low " " whole milk	0.5	0.5	1.0	2.0
High " " " "	0.0	0.0	0.5	0.5
Low " dry skimmilk	0.0	0.0†	0.0†	0.0†
High " " "	0.0	0.0†	0.0†	0.0†
Low " " whole milk	0.0	0.0	0.0	0.0
High " " " "	0.0	0.0	0.0	0.0

* Three p.p.m. copper added before freezing.
† A pronounced "powder" flavor present.

Effect of Adding to Fluid Milk Increasing Amounts of Condensed Whole Milk Upon Oxidized Flavor Development

Of all the concentrated milks investigated in this study, condensed whole milk and dried whole milk were the most effective as antioxidants when added to milk and ice cream. Since dry whole milk is often not available in fresh condition, it is possible that the condensed whole milk, although somewhat less effective, might have greatest application. It was decided to study the effect of adding a wider range of concentrations of condensed whole milk to milk in order to determine the smallest effective concentration and the effect of larger concentration on flavor. For this study homogenized whole milk from the College market milk department (contains about 0.3 p.p.m. of copper) was fore-warmed to 143° F. and concentrated to 34 per cent total solids and cooled over a surface cooler to 40° F. One half of this condensed was superheated to 185° F. for 3 minutes. This superheated condensed whole milk had an antioxidant effect similar to the unsuperheated product, but had the disadvantage in contributing a cooked flavor when used in the higher concentrations.

The low temperature condensed whole milk was added to raw milk in concentrations of 0.2, 0.6, 1.2, and 1.8 per cent milk solids-not-fat, pasteurized in glass flasks at 143° F. for 30 minutes and cooled to 40° F. Copper was added to the flasks with the agitators running. Table 9 shows the results of a representative series in which 0.5 p.p.m. of copper was added. Increased additions of the condensed whole milk effectively prevented the appearance of oxidized flavor, while lesser concentrations merely retarded

the flavor. The flavor of the milk was noticeably improved by the increased butterfat that accompanied the additions of 1.2 and 1.8 per cent milk solids-not-fat. In no case in this or other series when as high as two p.p.m. of copper was added did oxidized flavor appear when condensed whole milk was added at the rate of 1.2 and 1.8 per cent milk solids-not-fat.

TABLE 9

Effect of adding increasing amounts of condensed whole milk to fluid milk on oxidized flavor development

Amount of low temperature condensed whole milk added to raw milk before pasteurization	Degree of oxidized flavor after storage		
	1 day	2 days	3 days
Milk* plus 0.0% M.S.N.F.	2.0	4.0	4.0
" " 0.2% "	1.5	2.0	3.0
" " 0.6% "	0.0	0.2	0.2
" " 1.2% "	0.0	0.0	0.0
" " 1.8% "	0.0	0.0	0.0

* Five tenths p.p.m. copper added after pasteurization.

SUMMARY

As evidenced in these trials concentrated milk, either condensed or dried, may act as an antioxidant when added to fluid milk. The effectiveness of the concentrate in retarding oxidized flavor is influenced largely by the processing treatment given the concentrate, the type of product concentrated, the method of adding the concentrate and the amount of concentrate added. The relative ability of various types of concentrated milk to retard oxidized flavor in milk was correlated with a similar effect when used in ice cream.

High temperature treatments were not as effective in improving the antioxidant properties of the concentrated milk as was anticipated, but high temperature treatments after condensing, such as superheating the condensed milk, were more effective than similar temperatures applied to fluid milk in forewarming. Dried milk was more effective than the condensed milk from which it was dried. Condensing whole milk at a ratio of 2 to 1 concentration or greater improved its antioxidant properties.

Concentrated whole milk had a much greater antioxidant effect than concentrated skimmilk. This was true whether the comparison was made on M.S.N.F. or total solids basis.

If the concentrated milks were added to raw fluid milk before pasteurization, they had greater influence in retarding oxidized flavor than if added to the same milk after pasteurizing and cooling.

Additions of concentrated milk to fluid milk in amounts as little as 0.2 per cent milk solids-not-fat had a noticeable effect in decreasing the degree of oxidized flavor. As might be expected, increased additions of the concentrates had a greater retarding action. It was shown that an effective

antioxidant was produced in condensed whole milk which could be used advantageously at concentrations high enough to add 1.8 per cent total solids to fluid milk and 8.75 per cent milk solids-not-fat to ice cream without contributing any deleterious flavor.

REFERENCES

(1) BARNICOAT, C. R., AND PALMER, L. S. A Study of the Chemistry of Incipient Oxidation Defects in Butter. Minn. Agr. Expt. Sta. Tech. Bul. **134**. 1939.

(2) CORBETT, W. J., AND TRACY, P. H. Effect of Condensing on the Development of Oxidized Flavor. JOUR. DAIRY SCI., **23**, 3: 209. 1940.

(3) CORBETT, W. J., AND TRACY, P. H. Experiments on the Use of Certain Antioxidants for Control of Oxidized Flavor in Dairy Products. Food Res., **6**, 5: 445. 1941.

(4) DAHLE, C. D. Dry Skimmilk in Ice Cream. Ice Cream Field, **33**, 4: 15. 1939.

(5) DAHLE, C. D., AND FOLKERS, E. C. Factors Contributing to an Off-flavor in Ice Cream. JOUR. DAIRY SCI., **16**, 6: 529. 1933.

(6) DAHLE, C. D., WALTS, C. C., AND KEITH, J. I. Dry Skimmilk in Ice Cream. Pa. Agr. Expt. Sta. Bul. **271**. 1931.

(7) GOULD, I. A. A Study of the Reducing System of Milk. JOUR. DAIRY SCI., **23**, 10: 977. 1940.

(8) GOULD, I. A., AND SOMMER, H. H. Effect of Heat on Milk with Special Reference to the Cooked Flavor. Mich. Agr. Expt. Sta. Tech. Bul. **164**. 1939.

(9) JOSEPHSON, D. V., AND DOAN, F. J. Observations on Cooked Flavor in Milk—Its Source and Significance. Milk Dealer, **29**, 2: 35. 1939.

(10) KENDE, S. A Study of the Unknown Reducing Substances Capable of Causing Reduction in Milk and Their Influence on the Quality of the Milk and Its Derived Products. Proc. World's Dairy Cong., Sec. 2, p. 71. 1934.

(11) MUSHER, S. U. S. Patent No. 2,198,216. 1940.

(12) TROUT, G. M., AND GOULD, I. A. Homogenization as a Means of Stabilizing the Flavor of Milk. Mich. Agr. Expt. Sta. Quart. Bul., **21**, 1: 21. 1938.

STUDY OF SHORT-TIME–HIGH-TEMPERATURE PASTEURIZATION OF ICE CREAM MIX

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During the last few years there has been a renewal of interest in the pasteurizing of milk by the short-time–high-temperature methods. The writers since 1936 have studied various problems related to the development of one method of pasteurization of milk by a short-time–high-temperature process, commonly called the *Electro-pure*. Since many plants process both milk and ice cream mix, it would be desirable that the pasteurizer be capable of efficiently pasteurizing both products. Processing both products in the same equipment would have the following advantages: 1. Mixes of various compositions could be run consecutively. 2. The cost of cleaning and assembling equipment would be reduced because the mix could follow the milk through the short-time–high-temperature pasteurizer. 3. The complete processing of ice cream making might be streamlined by synchronizing the pasteurizer with the continuous freezer. 4. Overhead would be reduced by elimination of duplicate equipment.

In 1925 Martin, Swope and Knapp (6) used temperatures as high as 165° F. and holding periods from 15 minutes to two hours. They concluded that the flavor of the finished ice cream was slightly better when pasteurized at 145° or 155° F. than at 165° F.

Hening (4) in 1928 pasteurized ice cream mix at 180° F. for 10 minutes and found that the viscosity of the mix and the size of the fat clumps was decreased and that the whipping qualities were improved. However, the finished ice cream was criticized as having a cooked flavor and it was concluded that the use of 180° F. for 10 minutes was impractical.

Dahle, Keith and McCullough (2) vat pasteurized ice cream mix at 150° F. for 30 minutes, 160° F. for 20 minutes and 170° F. for 10 minutes and found that the mixes at the higher temperatures of pasteurization whipped faster and had a lower viscosity than did the mixes pasteurized at the lower temperature.

Dahle and Barnhart (1) used a continuous system of heating together with a centrifugal heater to secure momentary high temperatures in the pasteurization of ice cream mix. They found that ice cream mix so pasteurized at temperatures of 170° and 180° F. decreases the degree of fat clumping, reduces viscosity and freezing time and increases protein stability.

The pioneer work as reported by the above authors showed that ice cream mix could be pasteurized at high temperatures without injuring the properties of the mix with the exception of its flavor. In all of these studies a

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much longer holding time was used than is the common practice in the up-to-date short-time–high-temperature pasteurization equipment. In view of the improvement of such pasteurization equipment the results secured by the above investigators would not necessarily apply to present conditions.

The investigations reported herein were undertaken to determine the following:

1. Whether satisfactory commercial ice cream could be manufactured from mix pasteurized by the short-time–high-temperature method.

2. Whether ice cream mix should be homogenized before or following pasteurization.

3. To compare the quality of ice cream secured by the short-time–high-temperature method with that of the vat method of pasteurization of ice cream mixes.

METHODS EMPLOYED

Pasteurizers. 1. The short-time–high-temperature pasteurizer used was an Electro-pure unit manufactured by the Trumbull Electric Company, Plainville, Connecticut. The capacity of the pasteurizer for milk was 150 gallons per hour. For ice cream mix the capacity was lowered to 140 gallons per hour to synchronize it with the capacity of the homogenizer. The ice cream mix was drawn from a ballast tank through a York plate regenerator where it was heated from approximately 48° F. to 145° F. The mix was then pumped between two carbon electrodes where it was electrically heated to 180° F. and held in a holding chamber at this temperature for 19 seconds. From this point the mix flowed through a flow diversion valve and if the temperature was not up to 160° F. it was diverted to the raw supply; if the temperature was up to 160° F. it was forced through the regenerator and cooling plates. The mix was cooled by regeneration from 180° F. to about 85° F. and then by sweet water to 40° F.

2. The holder type pasteurizer was a standard spray E-100-gallon vat with a mix agitator manufactured by the Cherry-Burrell Corporation. This pasteurizer was equipped with a water temperature regulator and sentinel control. The pasteurization temperature used was 160° F. for 30 minutes. The mix was cooled over a cabinet surface cooler, using tap water in the top two sections and sweet water in the bottom section.

Homogenizer. The homogenizer used was a Manton-Gaulin model C.G.C.; a two-stage machine with a capacity of 125 gallons per hour. The homogenizer was equipped with a variable speed drive which was set at a capacity of 140 gallons per hour.

Freezers. The mix was frozen by a 60-gallon-per-hour Model JPV, Vogt Instant Freezer and a 40-quart-brine-batch Miller Freezer.

Processing the mix. One hundred gallons of an ice cream mix of the following composition were used: Fat, 14 per cent; serum solids, 12 per cent; sugar, 15 per cent; gelatin, 0.3 per cent.

The ingredients were raw four per cent milk, raw 40 per cent cream, skimmilk flakes, sugar and gelatin.

The raw milk and cream were placed in a mixing tank. The sugar, gelatin and skimmilk flakes were thoroughly mixed (dry) and added to the milk and cream with the agitator running. The mix entered the short-time–high-temperature pasteurizer at about 48° F., since heat was not applied to the mixing tank. Each 100-gallon batch of mix was then divided into four 25-gallon batches and processed as follows:

Lot A. The mix was drawn through the regenerator by the suction (vacuum) produced by the homogenizer, then homogenized, and forced by the homogenizer through the balance of the short-time–high-temperature pasteurizer. The mix was homogenized at the regeneration temperature of about 145° F. and then pasteurized at 180° F. for 19 seconds.

Lot B. The mix was drawn through the regenerator by suction (vacuum) produced by a Viking (positive) pump, and then forced through the electrode and holding chambers to the homogenizer. The mix was then homogenized at the pasteurizing temperature of 180° F. The homogenizer pumped the mix back through the cooling plates of the regenerator.

Lot C. The mix was heated to 160° F. in the vat, homogenized, and then pasteurized at 160° F. by the holding method for 30 minutes and cooled over a surface cooler.

Lot D. The mix was pasteurized in the vat at 160° F. for 30 minutes, then homogenized and cooled over a surface cooler.

The homogenization pressures used in all cases were 2000 pounds on the first stage and 500 pounds on the second stage valve.

Four five-gallon portions of mix from Lots A, B, C, and D were frozen in the following manner:

1. Frozen within four hours in a continuous freezer.
2. Mix aged 24 hours and then frozen in a continuous freezer.
3. Frozen within four hours in a batch brine freezer.
4. Mix aged 24 hours and then frozen in a batch brine freezer.

On each lot of mix the following data were obtained:

1. Standard plate counts before and after pasteurization were obtained on tryptone-glucose-skimmilk agar, prepared according to Standard Methods (7). The plates were incubated at 37° C. for 48 hours and counted according to the directions given in Standard Methods.
2. The efficiency of pasteurization was determined by a modification of the Gilcreas-Davis (5) phosphatase test.
3. The relative viscosity of the mix was obtained eight and 24 hours after pasteurization by determining the time required to drain one-half pint of ice cream mix through a Borden Flow Meter equipped with a glass tube, four mm. in diameter.

On each lot of finished ice cream data were obtained on:

1. Body and texture scores and criticisms were made by three judges working independently on duplicate samples of each lot of ice cream, keyed so that the judges did not know the identity of the samples. A score of 24.5 was given if the sample was considered perfect for body and texture. A score of 24.0 was allotted to samples which exhibited a very slight defect and a score of 23.5 for samples showing a definite defect.
2. The percentage of overrun was obtained by using the following formula:

$$\text{per cent overrun} = \frac{\text{Wt. of mix—Wt. of same volume of ice cream}}{\text{Wt. of same volume of ice cream}} \times 100$$

The average net weight of four pint packages of mix and the finished ice cream was used in the above formula.

RESULTS

The effect of processing on the bacterial count. The effect of the method of processing ice cream mix on the Standard Plate Count is shown in table 1. The average plate count of the mix prior to pasteurization was 305,000 per ml. The mix which was pasteurized by the short-time–high-temperature method after homogenization had an average plate count of 28 per ml. The

TABLE 1

The effect of the method of processing on the standard plate count of ice cream mix

Method of processing	Trial 1	Trial 2	Average
	colonies per ml.	*colonies per ml.*	*Colonies per ml.*
Raw (F)	114,000	690,000	
Raw (M)	85,000	800,000	305,000
Raw (L)	63,000	78,000	
Pasteurized			
A1 (F)	30	30	
A1 (M)	30	30	28
A1 (L)	30	20	
B2 (F)	40	60	
B2 (M)	30	20	36
B2 (L)	30	—	
C3 (F)	60	50	
C3 (M)	40	50	52
C3 (L)	100	10	
D4 (F)	710	50	
D4 (M)	80	80	188
D4 (L)	110	100	

— no sample taken.
(F) = first part of the batch; (M) = middle of the batch; (L) = last part of the batch.
A1 = Homogenized and short-time–high-temperature pasteurized.
B2 = Short-time–high-temperature pasteurized and homogenized.
C3 = Homogenized and pasteurized by the holding method.
D4 = Pasteurized by the holding method and homogenized.

mix which was pasteurized by the short-time–high-temperature method and then homogenized had an average plate count of 36 per ml. The mix which was homogenized and then holder-pasteurized had an average plate count of 52 per ml. while the mix which was holder-pasteurized and then homogenized had an average plate count of 188 per ml.

The data in table 1 indicate that both the holding and short-time–high-temperature methods of pasteurization were equally effective in reducing the number of bacteria. This does not substantiate the findings of Dahle and Knutsen (3) who reported that "the Electropure process of pasteurization proved to be more efficient in destroying bacteria than the holding method." The standard plate counts on the pasteurized mix were very low in all cases. Homogenization before or after pasteurization had no apparent effect on the number of bacteria destroyed.

The effect of processing on the phosphatase content. The effect of different methods of processing of ice cream mix on the phosphatase content is shown in table 2.

The data indicate that both short-time–high-temperature and holding methods of pasteurization were equally effective in the destruction of the

TABLE 2

The effect of the method of processing ice cream mix on the phosphatase content

Method of processing	Phenol value (mg. phenol/0.5 ml. of mix)					
	Trial 1			Trial 2		
	Sample	Boiled control	Net	Sample	Boiled control	Net
Raw (F)	2.48	1.20	1.28	2.26	1.06	1.20
Raw (M)	2.55	1.06	1.49	2.19	1.20	0.99
Raw (L)	2.42	1.06	1.36	.....	.....	.....
Pasteurized						
A1 (F)	0.0467	0.0484	−0.0017	0.0501	0.0450	0.0051
A1 (M)	0.0467	0.0723	−0.0256	0.0434	0.0450	−0.0016
A1 (L)	0.0467	0.0484	−0.0017	0.0410	0.0484	−0.0074
B2 (F)	0.0518	0.0434	0.0084	0.0401	0.0369	0.0032
B2 (M)	0.0426	0.0450	−0.0024	0.0377	0.0369	0.0008
B2 (L)	0.0476	0.0417	0.0059	.....	.....	.....
C3 (F)	0.0426	0.0536	−0.0110	0.0354	0.0369	−0.0015
C3 (M)	0.0442	0.0920	−0.0478	0.0385	0.0417	−0.0032
C3 (L)	0.0501	0.0467	0.0034	0.0385	0.0401	−0.0016
D4 (F)	0.0450	0.0484	−0.0034	0.0369	0.0401	−0.0032
D4 (M)	0.0442	0.0417	0.0025	0.0410	0.0385	0.0025
D4 (L)	0.0459	0.0434	0.0025	0.0354	0.0434	−0.0080

(F) = first part of the batch; (M) = middle of the batch; (L) = last part of the batch.

A1 = Homogenized and short-time–high-temperature pasteurized.

B2 = Short-time–high-temperature pasteurized and homogenized.

C3 = Homogenized and pasteurized by the holding method.

D4 = Pasteurized by the holding method and homogenized.

phosphatase enzyme and showed that the ice cream mixes were properly pasteurized. Homogenizing the mix before or after pasteurization had no apparent effect upon the phenol values obtained.

It should be pointed out that the phenol values of the boiled control were relatively high, which when subtracted from the phenol value of the sample gives a very low net phenol value. At times the phenol values were negative. At this time no explanation is offered for the negative phenol values.

The raw ice cream mix contained on the average 1.264 mg. phenol per 0.5 ml. of mix.

The effect of processing on the relative viscosity. The data in table 3 indicate that the relative viscosity of all lots of mix was low after aging 8 hours and also after aging 24 hours. The mix which was homogenized before short-time–high-temperature pasteurization had a slightly higher viscosity than did the other lots of mix, but the difference was not great enough to be significant. There was no significant difference in the viscosity of the mix holder-pasteurized and the mix short-time–high-temperature pasteurized. When the mix was holder-pasteurized there was no significant difference in the viscosity of the mix homogenized before pasteurization and the mix homogenized after pasteurization.

TABLE 3

The effect of the method of processing on the relative viscosity of the mix

Method of processing mix	Viscosity after 8 hours		Viscosity after 24 hours	
	Trial 1	Trial 2	Trial 1	Trial 2
lot	*seconds*	*seconds*	*seconds*	*seconds*
A	44	49	49	57
B	28	33	30	37
C	33	33	44	36
D	40	28	34	31

A = Homogenized and short-time–high-temperature pasteurized.
B = Short-time–high-temperature pasteurized and homogenized.
C = Homogenized and pasteurized by the holding method.
D = Pasteurized by the holding method and homogenized.

The effect of processing on the freezing and whipping ability. The data in table 4 indicate that the mixes aged 24 hours froze and whipped slightly faster than did the mixes which were aged for less than four hours but the difference was not great enough to be significant. The method of pasteurization or the time of homogenization had no apparent effect on the freezing time or the whipping ability of the mix.

The effect of processing and freezing on the overrun. Inconsistent results were obtained on the overrun secured on mix processed by different methods. This is shown in table 5.

Although an attempt was made to obtain an overrun of 85 per cent on

TABLE 4

The effect of the method of processing the mix on the freezing and whipping ability

Mix lot	Freezing time in minutes			Whipping time in minutes		
	Trial 1	Trial 2	Average	Trial 1	Trial 2	Average
A3	7	7	7.0	10	10	10.0
B3	5	7	6.0	10	9	9.5
C3	7	5	6.0	12	7	9.5
D3	6	6	6.0	9	8	8.5
A4	5	6	5.5	10	8	9.0
B4	5	5	5.0	8	8	8.0
C4	5	6	5.5	8	9	8.5
D4	6	7	6.5	8	9	8.5

Brine temperature was – 5° F. for trial 1 and – 2° F. for trial 2.

all mixes there was great variation in the overruns. This was undoubtedly due to the operation of the freezers rather than to the method of processing the mix. The data in trial 2 were more representative of the overruns which have been secured in later commercial trials.

The effect of processing on the body and texture. The data in table 6 show that the average body and texture scores for lots A, B, C, and D was 23.97, 23.90, 24.00, and 23.95, respectively. There was only 0.1 of a point difference in the average score between any of the four methods of processing the mix.

The average body and texture score of the ice cream frozen on the continuous freezer from unaged mix was 24.35, from aged mix, 24.30, while the

TABLE 5

The effect of the method of processing and freezing the mix on the overrun

Method of processing	Mix lot	Method of freezing	Per cent overrun	
			Trial 1	Trial 2
Homogenized and short-time–high-temperature pasteurized	A1	Continuous	54.5	76.5
	A2	Continuous	73.8	85.5
	A3	Batch	87.6	81.5
	A4	Batch	81.0	79.0
Short-time–high-temperature pasteurized and homogenized	B1	Continuous	55.5	87.2
	B2	Continuous	66.7	78.2
	B3	Batch	88.1	98.0
	B4	Batch	79.6	88.8
Homogenized and holder-pasteurized	C1	Continuous	56.9	80.2
	C2	Continuous	87.2	80.2
	C3	Batch	75.6	88.8
	C4	Batch	83.1	74.9
Holder-pasteurized and homogenized	D1	Continuous	39.9	82.6
	D2	Continuous	99.3	83.4
	D3	Batch	95.0	85.2
	D4	Batch	77.6	71.1

score for batch-frozen unaged mix was 23.55 and for aged mix, 23.70. The ice cream frozen in the continuous freezer scored 0.7 of a point higher than that frozen in a batch freezer. In all the trials the batch-frozen ice cream scored lower in body and texture than did the samples frozen on the continuous freezer regardless of the method of processing the mix or of the length of time the mix was aged.

The data in table 6 indicate that there is no significant difference in the body and texture of ice cream made from mix short-time–high-temperature pasteurized at 180° F. and mix holder-pasteurized at 160° F. Ice cream of equal quality as far as body and texture is concerned was made from mix homogenized either before or after pasteurization.

TABLE 6

SUMMARY OF TABLES

The effect of the method of processing the mix and the method of freezing on the body and texture

Mix lot	Method of processing the mix	Body and texture score, average
A	Mix homogenized and short-time–high-temperature pasteurized, 180° F.	23.97
B	Mix short-time–high-temperature pasteurized and homogenized, 180° F.	23.90
C	Mix homogenized and holder-pasteurized, 160° F.	24.00
D	Mix holder-pasteurized and homogenized, 160° F.	23.95
	Method of freezing the mix	
1	Continuous freezer; mix aged less than 4 hours	24.35
2	Continuous freezer; mix aged 24 hours	24.30
3	Batch freezer; mix aged less than 4 hours	23.55
4	Batch freezer; mix aged 24 hours	23.70

DISCUSSION

The Electro-pure pasteurizer used in these experiments was expressly designed for the pasteurization of milk at the rate of 150 gallons per hour. The final heating is accomplished by passing the milk between two carbon electrodes charged with electricity; the milk conducts the current from one electrode to the other. The resistance of the milk to the flow of the electric current causes the milk to be heated.

Before the trials reported herein were made, it was necessary to determine whether ice cream mix could be satisfactorily heated by the Electro-pure. It was found that milk, cream and sugar mixtures were not heated satisfactorily. However, when skimmilk flakes or its equivalent was added to the above mixture the conductivity of the mix was similar to that of whole milk so that the Electro-pure could be operated at 140 to 150 gallons per hour.

The preliminary experimental work reported herein was conducted in

April 1941 and since then 9,000 gallons of ice cream mix have been homogenized prior to short-time–high-temperature pasteurization. The commercial mix manufactured contained 12 per cent milk fat, 10 per cent serum solids, 15 per cent sugar, 0.4 per cent egg yolk and 0.2 per cent gelatin. The procedure for making this mix was as follows: the 40 per cent cream and 4 per cent milk were placed in a 100-gallon vat and agitated, the skimmilk flakes were then added and followed by about three-quarters of the total sugar. The remaining one-quarter of the sugar was mixed dry with the egg yolk and the gelatin before adding these ingredients to the mix. The mixes were pasteurized at 180° F., using the Electro-pure pasteurizer with a 19-second holding time. The mixes were drawn through the regenerator by the homogenizer and homogenized at 2,000 pounds pressure on the first stage and 500 pounds pressure on the second stage. The temperature of homogenization was about 140° F.

The only difficulty experienced during the year was that of excessive foaming of the cold mix in the mixing vat and in a few instances this foam plugged the pipe line between the mixing vat and the Electro-pure pasteurizer. The remedy used was to either heat the mix in the vat to 90° F. to break the foam or to apply hot water to the outside of the pipe line. In a permanent installation it would be advisable to place the mixing tank as close as possible to the Electro-pure pasteurizer to eliminate this difficulty.

The mix was processed immediately following the milk, thus saving labor of cleaning separate equipment for ice cream mix. No difficulty was experienced in obtaining the desired overrun of 90 per cent on the ice cream made from the mix so processed. The body and texture of the ice cream was very satisfactory.

After a year's trial in our commercial plant short-time–high-temperature pasteurization has been proven to be a satisfactory method of pasteurizing ice cream mix.

It should be pointed out that short-time–high-temperature method of pasteurization would not be satisfactory for butter mixes. In some trials frozen cream was used, but difficulty was experienced with "oiling off" prior to pasteurization.

SUMMARY

1. Pasteurization at 180° F. for 19 seconds proved as effective in reducing the number of bacteria in the ice cream mix as 160° F. for 30 minutes.

2. Homogenization before or after pasteurization does not affect the efficiency of bacterial reduction.

3. All samples of mix were considered properly pasteurized using the phosphatase test as a criteria.

4. The viscosity of all lots of mix was low and there was no significant difference between the lots homogenized before pasteurization and those homogenized after pasteurization.

5. The body and texture of the finished ice cream was more dependent on the method of freezing than on the method of pasteurizing the mix or the length of the aging time.

6. Ice cream mix when made from milk, cream and skimmilk flakes can be successfully pasteurized by a short-time–high-temperature pasteurizer.

7. No cooked flavor was noted in the finished ice cream pasteurized by either the short-time–high-temperature or the holder method.

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REFERENCES

(1) DAHLE, C. D., AND BARNHART, G. S. The Effect of Pasteurizing and Homogenizing Temperatures on Certain Properties of Ice Cream Mixes. Jour. Agr. Res., **42**, 10: 675. 1931.

(2) DAHLE, C. D., KEITH, J. I., AND MCCULLOUGH, A. D. The Effect of Aging the Mix on the Freezing Time, Overrun and Quality of Ice Cream. Pa. Agr. Expt. Sta. Bul. **247**: 20. 1930.

(3) DAHLE, C. D., AND KNUTSEN, M. H. Pasteurization of the Ice Cream Mix by Electricity. Pa. Agr. Expt. Sta. Bul. **213**: 21. 1927.

(4) HENING, J. C. Temperature of Pasteurization—How It Affects Milk Properties. Ice Cream Trade Jour., **24**, 10: 53. 1928.

(5) HIERL, V. H. Some Variations in the Phosphatase Activity of Cow's Milk. A Thesis, Univ. of Conn. 1941.

(6) MARTIN, W. H., SWOPE, W. D., AND KNAPP, I. R. Problems in the Manufacture of Ice Cream—Pasteurization Temperatures. Pa. Agr. Expt. Sta. Ann. Rpt. 38 (Bul. **196**): 20. 1925.

(7) Standard Methods for the Examination of Dairy Products. Amer. Pub. Health Assoc., 8th Ed. 1941.

GROWTH STUDIES WITH AYRSHIRE CATTLE. I. NORMAL BODY WEIGHTS AND HEIGHTS AT SHOULDERS FOR AYRSHIRE CATTLE[1]

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The normal growth of dairy cattle has been of primary importance as a complementary aid in dairy cattle research for many years. While this interest was first related almost entirely to nutritional studies, within recent years it has been of value in the field of animal breeding in determining the normal growth of cattle in registered herds. Among the experiment stations having herds of Ayrshire cattle, only a few (1, 2, 3, 4, 5) have reported normal growths for Ayrshires, and the numbers involved in each study have been relatively small. The West Virginia Agricultural Experiment Station, because of its ownership of the Lawrence A. Reymann Memorial herd, has had an opportunity to collect extensive data relative to the normal growth of this breed. These data have been assembled and tabulated with the idea that they may be of real interest and assistance to students of dairy cattle breeding. This paper is the first of a series based on the analysis of the growth data gathered in the Reymann Memorial herd.

PROCEDURE

The Reymann Memorial herd was originally located at Wardensville, West Virginia, 140 miles from the Experiment Station, and the study was begun at that location. Following the destruction of the milking barns by fire in August, 1937, the milking herd was moved to the Experiment Station farm at Morgantown. The heifers, however, after being weaned, have been taken for raising to the Reymann Memorial Farms and then returned to the University herd just previous to calving. This management routine has assured practically the same environmental conditions for the heifers, as far as conditions could be controlled, throughout the entire study.

The collection of growth data was started in January 1927 and has been continued without interruption. First weights of the calves were taken within 24 hours of birth. Body weights and height-at-shoulder measurements were taken at monthly intervals. When measuring heights three measurements were taken, the animal being moved before each measuring, and the three figures averaged. All measurements were recorded in centimeters and all weights were recorded in pounds. Data were collected on 242 females and 165 males. In a few cases, due to oversight on weighing

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TABLE 1

Body weights and heights at shoulders by months of Ayrshire females in the Reymann Memorial herd of the West Virginia Agricultural Experiment Station

Body weight			Height at shoulders		Body weight			Height at shoulders	
Age (months)	Number animals	Weight (pounds)	Number animals	Height (inches)	Age (months)	Number animals	Weight (pounds)	Number animals	Height (inches)
At birth	194	70	.....	.....	55	57	1129	57	50.5
1	188	84	169	27.2	56	56	1143	56	50.5
2	228	111	215	28.8	57	55	1154	55	50.5
3	237	146	227	30.8	58	55	1165	55	50.4
4	242	184	231	32.6	59	53	1158	53	50.5
5	242	227	232	34.6	60	50	1157	50	50.4
6	242	272	233	36.0	61	54	1157	54	50.2
7	242	318	236	37.2	62	47	1149	47	50.1
8	242	361	235	38.5	63	46	1180	46	50.2
9	241	401	234	39.6	64	46	1164	46	50.3
10	242	440	235	40.6	65	45	1165	45	50.3
11	242	475	236	41.4	66	44	1189	44	50.5
12	240	506	235	42.2	67	44	1190	44	50.5
13	242	531	236	42.8	68	42	1208	42	50.5
14	242	556	236	43.3	69	38	1197	38	50.7
15	242	584	236	43.8	70	36	1192	36	50.7
16	242	616	236	44.4	71	35	1196	35	50.8
17	242	642	236	44.9	72	33	1182	33	50.8
18	241	674	236	45.3	73	33	1183	33	50.8
19	242	697	236	45.8	74	32	1187	32	50.6
20	242	721	237	46.1	75	32	1203	32	50.7
21	242	745	237	46.5	76	32	1242	32	50.5
22	241	768	235	47.1	77	32	1216	32	50.7
23	241	793	237	47.2	78	33	1251	33	50.7
24	242	819	237	47.5	79	29	1267	29	50.8
25	240	852	237	47.7	80	26	1273	26	50.9
26	239	868	236	48.0	81	23	1248	23	51.1
27	242	901	239	48.3	82	21	1224	21	51.0
28	234	928	234	48.6	83	18	1186	18	50.9
29	230	948	231	48.6	84	17	1164	17	50.8
30	218	970	218	48.7	85	20	1178	20	50.6
31	212	995	212	49.0	86	20	1187	20	50.6
32	202	1006	202	49.2	87	19	1184	19	50.6
33	187	1016	187	49.2	88	18	1170	18	50.6
34	173	1022	173	49.4	89	18	1199	18	50.6
35	159	1017	159	49.4	90	17	1225	17	50.6
36	152	1023	152	49.6	91	17	1256	17	50.5
37	146	1031	146	49.7	92	17	1281	17	50.6
38	143	1041	143	49.8	93	16	1245	16	50.4
39	136	1047	136	49.9	94	16	1220	16	50.5
40	130	1052	130	49.9	95	14	1208	14	50.5
41	128	1067	128	50.0	96	12	1176	12	50.5
42	120	1081	120	50.0	97	15	1177	15	50.6
43	114	1096	114	50.0	98	15	1167	15	50.8
44	100	1110	100	50.1	99	14	1169	14	50.6
45	87	1130	87	50.3	100	12	1184	12	50.6
46	72	1147	72	50.3	101	12	1206	12	50.3
47	61	1159	61	50.6	102	11	1213	11	50.5
48	45	1173	45	50.5	103	9	1217	9	50.3
49	59	1113	59	50.3	104	9	1227	9	50.3
50	59	1107	59	50.3	105	9	1196	9	50.3
51	59	1110	59	50.3	106	9	1208	9	50.1
52	59	1129	59	50.3	107	8	1196	8	50.3
53	58	1108	58	50.3	108	8	1212	8	49.9
54	57	1133	57	50.5					

days, weights or measurements were not taken on individuals, resulting in a variation from month to month in the total number of animals involved.

Animals involved in this study were fed a normal grain and hay ration during the feeding periods and had access to bluegrass pasture during the grazing season.

RESULTS

A summary of heights at shoulders and body weights for Ayrshire females is shown in table 1. These data are shown on a monthly basis, and they cover ages from birth to 108 months, or nine years. The number of animals at each age interval and the average body weights are included in this table. Body weight and height data for Ayrshire bulls are shown in table 2.

The ranges in body weights and heights at shoulders for Ayrshire females are shown in figures 1 and 2.

TABLE 2

Body weights and heights at shoulders by months of Ayrshire males in the Reymann Memorial herd of the West Virginia Agricultural Experiment Station

	Body weight		Height at shoulders	
Age (months)	Number animals	Weight (pounds)	Height (inches)	Height (cms.)
At birth	165	76	.....	.....
1	88	88	27.4	69.5
2	96	120	29.3	74.5
3	89	166	31.7	80.5
4	79	212	33.4	84.9
5	67	269	35.5	90.2
6	67	327	37.4	94.9
7	60	387	39.0	99.1
8	57	439	40.2	102.2
9	55	488	41.4	105.2
10	47	536	42.1	107.0
11	42	573	43.1	109.5
12	37	621	43.8	111.2
13	32	657	44.5	113.0

DISCUSSION

Comparisons of the data for Ayrshire females with those collected at the Missouri, Iowa and Kansas stations indicate certain differences which may be attributed to variations due to (a) numbers of animals included in the studies, (b) environmental conditions, and (c) intra-breed differences.

The Missouri data (2), commonly known as the *Eckles Normal*, included only 26 birth weights and weights on a maximum of 12 females at any age. The birth weights of the Missouri animals were practically the same as for the Reymann Memorial females but the monthly weights and heights at shoulders exceeded the Reymann Memorial cattle until the sixth and seventh months, respectively. After these ages they definitely fell below the heights

and weights of the Reymann memorial animals as long as the data were collected. Such behavior may well indicate a more liberal feeding regime at the Missouri Station which was offset by an intra-breed difference as the animal developed, for at maturity the Missouri females were definitely smaller than the Reymann Memorial females.

Ayrshires in the Kansas Experiment Station (4) herd, while only slightly heavier than the Reymann Memorial females at birth, were definitely taller,

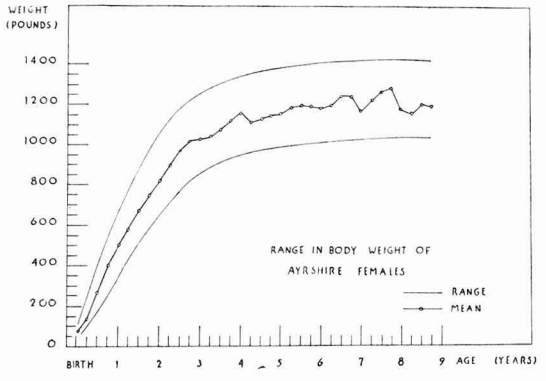

FIG. 1.

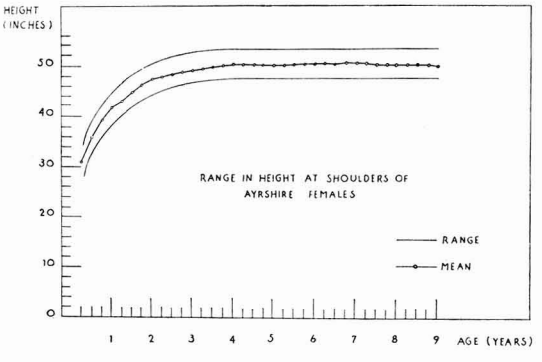

FIG. 2.

and as age increased they maintained a definite superiority in height at shoulders. This would indicate an intra-breed difference as compared to the Reymann Memorial Ayrshires. At two years of age the Kansas animals were also definitely heavier.

The Iowa heifers (3) check closely with the Reymann Memorial heifers in heights at shoulders until they are two years of age, after which the heifers in the Iowa herd are on the average lower in height throughout the remainder of the curve. In body weights the heifers represented in the

Iowa data weighed less than Reymann Memorial heifers until they were four months of age, and then exceeded the Reymann Memorial animals in body weight until 30 months of age. After 30 months of age the Reymann Memorial females exceeded the Iowa females in body weight throughout the remainder of the curve.

The differences in the heights and weights of bulls in the Reymann Memorial herd as compared to the bulls in the Kansas Station herd may again indicate an intra-breed difference, but since no mature weights were available definite conclusion cannot be drawn.

The mature weights and heights of Ayrshire females as reported from data collected in the Reymann Memorial herd and compared to data collected at Missouri and Iowa would indicate an intra-breed difference due to the presence of different strains of the breed exerting dominant influences in the respective herds. The dangers of making generalizations regarding normal weights and heights for the breed from data definitely influenced by individual strains has been pointed out by Espe, Cannon and Hansen (3). It is hoped that because of the size of the Reymann Memorial herd (approximately 120 head), and the fact that many bulls from different families and strains have been used, the influence of any strain which might introduce into the herd an inheritance for abnormal heights or body weights would be reduced to a minimum.

Figures 1 and 2 bring out clearly the differences in the variations in heights and body weights of Ayrshire cattle. Between the tallest and lowest mature cows there was a difference of only 5.7 inches, or a coefficient of variation of only 3.1 per cent. In body weights, however, the difference between the extremes was 400 pounds, or a coefficient of variation of 13.03 per cent.

Table 1 as well as figures 1 and 2 show that while Ayrshire cattle increase steadily in body weight until they reach productive maturity, very little increase is made in height at shoulders after the animals reach three years of age—facts which should be worthy of consideration in appraising animals of various ages when evaluating results in breeding programs, or in judging or classifying animals in the show-ring.

Some of the factors influencing the growth of Ayrshire cattle in the Reymann Memorial herd will be presented in future manuscripts.

SUMMARY

Birth weights, monthly body weights and monthly height-at-shoulders measurements are reported for Ayrshire females to 9 years of age, and males to 13 months of age in the Reymann Memorial herd of the West Virginia Agricultural Experiment Station. Comparisons are made between these data and similar data published by the Missouri, Iowa and Kansas Experiment Stations. The percentage variations between the extremes in body weights, and between the extremes in height measurements are also noted.

ACKNOWLEDGMENTS

The authors are indebted to Professor F. D. Cornell and his assistants in the Department of Agricultural Economics for tranferring the growth data to punch cards, and for making the necessary sortings. The authors also acknowledge the assistance of Mr. R. A. Ackerman of the Reymann Memorial Farms in the collection of the data.

REFERENCES

(1) DAVIS, H. P., MORGAN, R. F., BRODY, SAMUEL, AND RAGSDALE, A. C. Relation of Height at Withers and Chest Girth to Live Weight of Dairy Cattle of Different Breeds and Ages. Neb. Agr. Expt. Sta. Res. Bul. **91**. 1937.

(2) ECKLES, C. H. The Normal Growth of Dairy Cattle. Mo. Agr. Expt. Sta. Res. Bul. **36**. 1920.

(3) ESPE, D. L., CANNON, C. Y., AND HANSEN, E. N. Normal Growth in Dairy Cattle. Iowa Agr. Expt. Sta. Res. Bul. **154**. 1932.

(4) FITCH, J. B. Normal Growth of Dairy Cattle. Mimeographed Report, Amer. Dairy Science Assoc., Annual Meeting, 1932.

(5) RAGSDALE, A. C. Growth Standards for Dairy Cattle. Missouri Agr. Expt. Sta. Bul. **336**. 1934.

THE PREPARATION OF CRYSTALLINE RENNIN*

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Rennin has been the subject of many papers, but the evidence characterizing the chemical and physical properties of the enzyme has been highly contradictory. Conflicting views have arisen with regard to the mode of action of rennin possibly because various crude preparations as well as so-called "pure" preparations have been used. It is obviously of essential importance to obtain rennin in its highest state of purity for investigational work involving studies of the enzyme itself, its action, or the resulting product of the enzyme action.

A method for the purification of rennin from commercial extract and some of the properties of the purified product were described in a recent paper by Hankinson and Palmer (2). Attempts to crystallize the highly purified protein were unsuccessful.

The crystallization of rennin was recently accomplished, however, in the course of experimentation with procedures to give a further purification with decreased losses of active material. A report of these experiments, including the relation of several recognized variables and some of the properties of crystalline rennin, is the purpose of this paper.

The above-mentioned paper may be referred to for a review of the more recent attempts to purify rennin. As has been pointed out in this review, an evaluation of the purity or relative activity of the various preparations is practically impossible because of failure to state conditions for measuring activity as well as failure to adopt any uniform procedure or standard rennin solution for comparing activities.

One additional reference, which was found in the patent literature and was unintentionally overlooked in the aforesaid review, is of interest. This patent by Keil and Stout (3) describes a process of preparing rennin whereby the pH of their extract is adjusted to 4.5 and the suspension is saturated with sodium chloride. These conditions are somewhat similar to those which we found independently, but at about the same time, to be optimum for our first precipitation from the commercial rennet extract. However, the precipitate obtained by this one fractionation is far from being pure rennin; three or four fractionations are usually necessary to remove the major impurities.

EXPERIMENTAL METHODS

Purification procedure. The procedure which has been found to be the

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most satisfactory, as a result of twenty-five comprehensive fractionation experiments, is as follows:

The pH of the rennet extract is adjusted to approximately 5.0 with concentrated hydrochloric acid. Sodium chloride is then added to the solution until it is saturated to "salt out" the rennin. The suspension is centrifuged for one-half to one hour at 2000 r.p.m. and the supernatant liquid containing the major portion of non-rennin material is decanted from the active rennin precipitate. The non-rennin material consists of pepsin, soluble protein, non-protein nitrogenous material, inorganic salts, and a red-brown colored material.

The precipitate is dispersed in one-half the original volume of water and dissolves upon adjusting the pH to 5.7 to 6.0. The fractionation is repeated until the fourth precipitate is obtained, dissolving the precipitate in one-half the preceding volume at pH 5.7 to 6.0 each time.

The fourth precipitate is dispersed in water and dialyzed about 24 hours in a cellophane membrane against running distilled water until practically free from sodium chloride. The dialyzed suspension is diluted to approximately 0.05 per cent solids concentration, adjusted to pH 5.7 to 6.0, and filtered, using suction to remove any insoluble material. Toluene should be added as a preservative at this stage if considerable time is consumed in carrying out the purification and crystallization procedures at room temperature.

Crystallization procedure. The pH on the above solution is adjusted slowly with N/10 HCl, accompanied by slow stirring, until the first definite turbidity is obtained. It is allowed to stand about ten minutes and enough more acid is added to decrease the pH about 0.1 unit. More acid may be added at approximately ten-minute intervals until a heavy white turbidity but no flocculation is obtained. Upon examination of a drop of the liquid under the microscope, many white needle-shaped crystals are observed. The crystalline suspension is allowed to stand several hours at room temperature and then centrifuged. The supernatant liquid may be further acidified and more crystals removed until a pH of 4.5 is reached.

Recrystallization may be effected by repeating the crystallization procedure but this has been found to be unnecessary, because of losses of active material with no further increase in activity per unit weight. The crystals are dissolved and preserved in the cold in a small volume of 20 per cent sodium chloride (by volume) at pH 5.7 to 6.0.

Care should be taken in the crystallization procedure to prevent precipitation of amorphous material which may result from the following:

(1) Too rapid addition of acid.
(2) Solids content greater than 0.05 per cent.
(3) Insufficient purification prior to crystallization.
(4) Crystallization temperature greater than 20 to 25° C.

In some experiments where difficulty was encountered with amorphous material in the crystallization procedure, it was found that a slower crystallization employing refrigerator temperatures, or crystallization from a dilute sodium chloride solution by using a mixture of equal parts of N/10 HCl and NaCl to adjust the pH were more successful.

Determination of rennin activity. Clotting activity was determined by pipetting 10-ml. portions of fresh raw whole milk into suitable test tubes in a constant-temperature water bath held at 40° C., allowing five minutes to come to temperature, then adding 0.5 ml. of the rennin sol at several dilutions, allowing one-half minute between samples, and recording the coagulation time. For comparing activity of several rennin samples, a final trial was made on all the samples simultaneously, using only the dilutions which gave a clotting time approaching ten minutes in the preliminary trial. Actual coagulation times were estimated by stop watch to the closest five seconds.

The activity of each sol was calculated on the basis of the sodium-chloride-salt-free dry weight of a dialyzed aliquot necessary to produce coagulation in ten minutes as compared to the standard. This was accomplished by dialyzing about 50 ml. of each sample over night in cellophane tubings ($\frac{3}{4}$ inch in diameter) against running distilled water, recording the volume change, making a solids determination and determining the residual sodium chloride, using standard $AgNO_3$ solution and K_2CrO_4 indicator. Both the relative activity per unit weight and the total activity could be followed in this way.

EXPERIMENTAL RESULTS

The results of a typical fractionation (Experiment 19) are outlined in table 1. The starting material was a portion of a ten-gallon lot of special rennet extract kindly furnished by the Chr. Hansen's Laboratories for this

TABLE 1

Outline showing relative activities and losses in purification

Fraction	Rel. act. / Unit wt.	Total wt.*		Total activity
		gm.	*per cent*	*per cent*
1. Original (4 l.)	1.00	45.20	100.0	100.0
2. 1st sup.	0.22	23.42	51.8	11.4
3. 1st ppt.	1.97	18.08	40.0	78.8
4. 2nd sup.	0.81	7.48	16.6	13.5
5. 2nd ppt.	3.54	7.69	17.0	60.2
6. 3rd sup.	0.94	1.20	2.7	2.5
7. 3rd ppt.	4.16	6.24	13.8	57.4
8. 4th sup.	0.87	0.56	1.2	1.0
9. 4th ppt.	4.58	5.53	12.2	55.9
10. Crystalline	4.70	2.63	5.8	27.3

* The weight and total activity values are corrected for aliquots removed for dialysis.

TABLE 2

Relative activities of several commercial and purified preparations

Preparation	Number of samples	Salt-free dry weight (dialyzed)	Nitrogen by vol. (undial.)	Rel. act. / Vol.	Rel. act. / Dry wt. (dial.)	Rel. act. / Wt. nitrogen
		per cent	*per cent*			
1. Commercial extract*	3	1.26	0.571	1.00	1.00	1.00
2. Commercial extract dialyzed	1	0.91	0.128	0.67	0.93	2.97
3. Special extract*	8	1.13	0.520	1.17	1.31	1.29
4. Rennet powder 10% solution*	1	1.02	0.202	2.15	2.65	6.08
5. Rennet powder 2% solution†	1	0.72	0.132	0.81	1.42	3.48
6. Rennet powder 10% solution‡	1	0.92	0.138	0.74	1.01	3.06
7. Crystalline preparation (Exp. 19)	1	.....	.....	.....	6.16	18.5
8. Crystalline preparations	6	.....	.....	.....	5.98	18.0 (calc.)
9. Crystalline preparations of highest activity	2	.....	.....	.....	6.96	21.0 (calc.)
10. Purified preparations not crystallized	6	.....	.....	.....	5.32	16.0 (calc.)

* By courtesy of Chr. Hansen's Laboratory, Inc.
† By courtesy of Armour and Company.
‡ Prepared by method of Keil and Stout.

work. It consisted of a specially prepared liquid rennet made by extracting with pure salt brine.

It is shown in table 1 that 27.3 per cent of the original activity was concentrated in 5.8 per cent of the original weight, thus making the crystalline product 4.70 times the original on a dialyzed-dry-weight basis. It will be shown later in table 2 that the special rennet extract is 1.31 times as active as commercial rennet extract on a dialyzed-dry-weight basis, thus making the activity of the crystalline product approximately six times that of the commercial extract.

The relative activities of several commercial and purified preparations are shown in table 2. It should be noted that dialysis increases the relative activity about two to three times; thus fifty to sixty-seven per cent of the

TABLE 3

Preliminary analysis of several pure rennin preparations

Constituent	Content	Method
	per cent	
C	51.40	Micro-combustion
H	7.19	Micro-combustion
N (total)	14.51	Dumas
N (total)	15.05	Kjeldahl
N (free amino)	1.11	Gasometric—Van Slyke
S	1.46	Parr bomb—benzidine*
S	1.46	Parr bomb—$BaSO_4$*
P	0.041	Spectrophotometric†
Fe	None	Thiocyanate
Cu	0.0035	Dithizone
Total Ash	0.2	Usual

The C, H, and N (Dumas) determinations were made by E. Renfrew, Chemistry Department, University of Minnesota, on a portion of the crystalline fraction of Experiment 19 (see table 1), dried at 102° C. They were corrected for 1.4 per cent NaCl.

The author is indebted to M. Ellertson, V. Nelson, W. Gleim and D. Smith of this laboratory for assistance with the analytical phase of this work.

* Callan and Toennies, Ind. Eng. Chem., Anal. Ed., **13**: 452. 1941.

† Goodloe, Ind. Eng. Chem., Anal. Ed., **9**: 527. 1937.

total nitrogen in the starting materials exists as low molecular weight, dialyzable, non-rennin-active substance. The special rennet powder, Hansen's 30X, was the most highly "pure" starting material studied, being about twice as active on a dialyzed-salt-free-dry-weight and total-nitrogen basis as either the Armour special rennet powder or the rennet powder prepared by the author according to the method of Keil and Stout. This statement should not be misconstrued to mean the relative activity on a unit weight or volume of the various commercial products as they exist, containing a variable content of sodium chloride. The degree to which any sample may have been diluted or concentrated with respect to either sodium chloride or water has no significance in this study.

The crystalline rennin is shown in table 2 to have six to seven times the activity of the commercial rennet extract on a dialyzed-salt-free-dry-weight basis and 18 to 21 times the activity on a nitrogen basis.

A preliminary study of general chemical analysis of pure rennin is summarized in table 3. It is interesting to note the sulfur content of 1.46 per cent. This is higher than the usual sulfur content of proteins and possibly may be significant when the exact mechanism of rennin action on casein is understood. The evidence accumulating recently on the role of sulfur in the structure of wool proteins further points to this possibility.

It is questionable whether the 0.041 per cent phosphorus is present as an actual constituent of the rennin molecule or is present as an impurity. The same doubt exists about the trace of copper found. This can only be

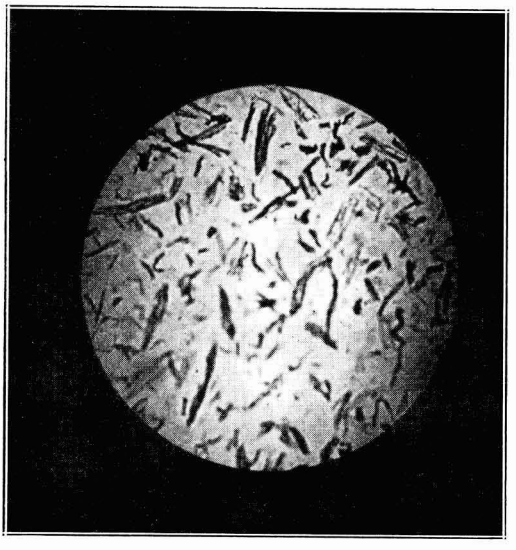

FIG. 1. Typical microscopic field of rennin crystals. 100×.

settled when more material has been obtained for several recrystallizations followed by electrodialysis.

The iron analysis was included because the relatively inactive reddish brown precipitate which is formed in the zone of pH 6.0 to 6.5 was found to possess a significant amount of iron. This precipitate was assumed to be hemoglobin present in small amounts in the calves' stomachs and extracted along with the rennet. As is shown in the table, pure rennin is free from iron.

Figure 1 shows a photomicrograph of a typical field of rennin crystals. Considerable difficulty was encountered in securing satisfactory photographs. The dark-field apparatus shows the crystalline shape and detail to better advantage, but no satisfactory photomicrographs were obtained. The crystals always appear as spherical blurs on the photographic film at a wide

variation of time and light intensities. The crystals were discovered to be dissolving slowly when placed on a glass slide under the microscope, some fields of crystals completely disappearing in five minutes. A cover slip increased the permanency somewhat. The phenomenon did not appear to be due to a simple heating effect nor to light sensitivity, because crystalline suspensions in test tubes held at 37° C. or under a 60-watt electric light bulb at room temperature for one-half hour did not appear to lose any crystals. This slow erosion together with brownian movement of the small crystals possibly accounts for the poor dark-field photographing results. A satisfactory

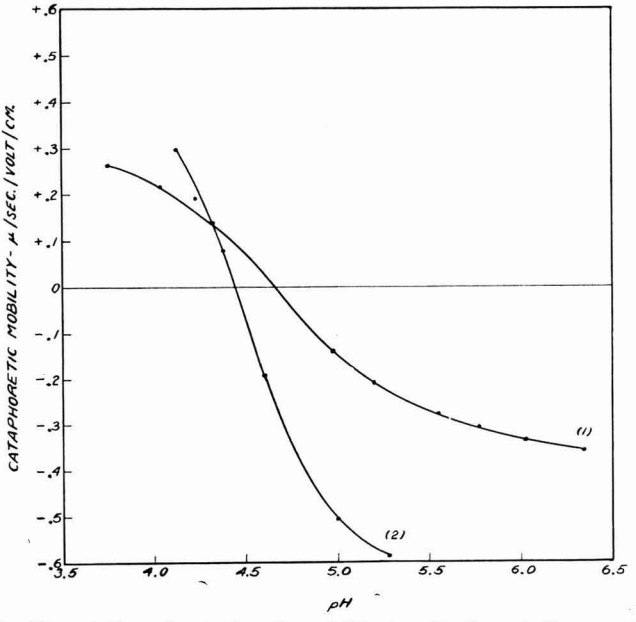

FIG. 2. The relation of cataphoretic mobility to pH of crystalline rennin suspensions. Curve (1)—Exp. 20. 0.056% rennin, 0.017 M NaCl. Curve (2)—Exp. 21. 0.048% rennin, 0.0007 M NaCl.

explanation of the disappearance of the crystals under the microscope is not apparent at present to the author.

The isoelectric point of rennin was determined by the cataphoretic mobility method, using the apparatus described by Briggs(1). Two different crystalline preparations were used and no attempt was made to use identical concentrations of protein and sodium chloride in the two experiments. All these factors may contribute to the difference in the two curves shown in figure 2. The isoelectric point of the 0.056 per cent rennin in 0.017 M NaCl of Experiment 20 is shown to be approximately 4.65. The isoelectric point of the 0.048 per cent rennin in 0.0007 M NaCl appears

to be approximately 4.45. A more detailed study would be necessary to establish the exact isoelectric point of rennin under various conditions. The isoelectric point was determined in this study to decide the pH at which maximum precipitation or crystallization could be expected from large volumes of dilute rennin solutions.

DISCUSSION

The general principles discussed by Northrup (4) for preparation and crystallization of the enzymes proved to be valuable in the present work. However, better results have been obtained by the author in rennin purification by centrifuging rennin suspensions rather than by filtering them by suction, because of the coagulum character of the precipitate formed. The principle of dialysis, which Northrup failed to emphasize, was found to be highly effective in this work for removing troublesome impurities of small molecular weight. Many adsorbing agents were tried but none was found practical, because those which removed impurities also removed a high proportion of rennin, due to its extremely high surface activity. When tried for the purpose of preferentially adsorbing rennin, the losses of active material were too great.

The various experiments leading to the results reported in this paper demonstrated to the author the importance of controlling several variables for reproducible results in the fractionation procedure. These may be listed as: 1) protein concentration, 2) salt concentration, 3) pH, 4) kind of salt, and 5) conditions of dialysis. Two additional variables of importance in the crystallization procedure are: 1) temperature, and 2) time.

Methods must be used in rennin purification which prevent loss in activity due to: 1) microorganisms natural to the rennet extract or introduced by contamination, 2) pepsin, 3) alkalinity, 4) high acidity which favors peptic action, and 5) drying. A high salt concentration, temperatures between 0° and 25° C., and toluene have been used effectively to control destruction due to microorganisms. Limiting the pH zone to 4.5 to 6.5 and using low temperatures minimizes the destruction due to pepsin, alkalinity and acidity. Preservation of samples in salt solution or under toluene in the cold has made it unnecessary to dry samples in this study, except for an aliquot for solids determination or for chemical analysis. Evaporation from the frozen state may be resorted to when the active dry material is desired.

Preliminary work established the practicality of using Hansen's commercial rennet extract as a standard solution and expressing all activities as relative activities in this work. Considerable precaution is evidently taken by the manufacturers of this product in standardizing the activity and stability of different lots of extract, because the activity of new lots of rennet usually checked within five to ten per cent when compared with previous lots stored several months in the refrigerator at 5° to 10° C.

The variability of milk itself and the multiplicity of variables influencing rennin activity have been limiting factors in establishing an absolute measure of rennin activity. The adoption of the relative activity method for measuring the degree of purification was found to minimize the importance of these variables provided that they were held constant for any one experiment. The relative activity of a purified preparation with respect to the standard rennet extract remained practically constant when the source of milk, time of coagulation, and concentration of added $CaCl_2$ or HCl were varied over a rather broad range. Therefore natural raw milk was used even though the conditions adopted for determining rennin activity were not optimum with respect to pH or calcium ion concentration.

Irregularities occasionally occurred in comparing activities by the method described. It was noted that they usually appeared when dialysis was not very complete, as though some non-rennin constituent other than sodium chloride was dialyzable. Nitrogen determinations revealed a relatively high concentration of dialyzable nitrogenous matter present in varying proportions in the different fractions. Several trials showed no loss of clotting activity when carried out in the above manner, so this dialyzable nitrogen was either present in the original extract or resulted due to degradation of some non-rennin material during dialysis.

As a consequence of this finding, a more accurate and simplified method of comparing activities in the later experiments was found to be on the basis of relative milk clotting activity per unit weight of nitrogen. The dialysis procedure, chloride and solids determinations were unnecessary by this method and thus reduced sources of error.

SUMMARY

A method is presented for the purification and crystallization of rennin. The several variables and precautions involved are discussed in some detail.

Crystalline rennin shows a relative activity of six to seven times the commercial rennet extract on a dialyzed-salt-free-dry-weight basis and 18 to 21 times on a total nitrogen basis. A high concentration of non-rennin-active, dialyzable, low molecular weight nitrogenous material, amounting to about two-thirds of the total nitrogen was separated from the rennin by either dialysis or salting out procedures. The relative activities of several commercial and purified preparations are compared.

Some of the general chemical properties of the crystalline product are presented. Attention is directed to the high sulfur content of 1.46 per cent, the absence of iron and questionable contents of phosphorus and copper. The isoelectric point was found to be in the zone of pH 4.45 to 4.65.

It is pointed out that some of the studies reported on crystalline rennin should be recognized as preliminary in nature. The availability of larger amounts of crystalline product should establish more definite values and

fundamental knowledge concerning the chemical nature and mode of action of the enzyme.

REFERENCES

(1) BRIGGS, DAVID R. A Pyrex All-Glass Microelectrophoresis Cell. Indus. and Engin. Chem., Analyt. Ed., **12**: 703–705. 1940.

(2) HANKINSON, C. L., AND PALMER, L. S. Purification of Rennin from Commercial Rennin Extract: Properties of the Purified Product. JOUR. DAIRY SCI., **25**: 277–283. 1942.

(3) KEIL, HAVARD L., AND STOUT, BETTY K. Process of Preparing Rennin. U. S. Patent No. 2,145,796. Patented Jan. 31, 1939.

(4) NORTHRUP, JOHN H. Crystalline Enzymes, p. 127. Columbia Univ. Press, New York. **1939.**

COMPARATIVE STANDARDIZATION OF BUTTER, CHEESE, MILK AND ICE CREAM FLAVOR SCORING

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For some time the belief has been quite common among coaches of dairy products judging that the scoring of flavor defects of butter and cheese has been well standardized, while considerable doubt has existed as to the extent of standardization in the scoring of those defects of milk and ice cream. Unquestionably, marked progress has been made in the judging of dairy products during the past twenty years. Despite this improvement, however, the feeling has persisted that dairy products judges do a better job at judging butter and cheese than they do at judging milk and ice cream.

In order to arrive at more uniform scoring throughout the country through establishment of standards, an attempt has been made to retain the same official judge for the respective product year after year in the Students' National Contest in the Judging of Dairy Products. As a result Messrs. Edwards, Wilson, Babcock, and Dahlberg have acted as official judges of butter, cheese, milk, and ice cream, respectively, with few exceptions, for the past ten years.

The beneficial influence of this continuity of judgment by the same judges year after year on dairy products judging throughout the United States is immeasurable. Each year coaches of dairy products judging teams have looked forward to checking their judgments with those of the officials, thus being able to carry back to their respective states a mental standard of quality for each product. Consequently, standardization of scoring to certain levels was inevitable. Although marked improvements have been made, there apparently exists an opportunity for further standardization of the judging of dairy products.

Inasmuch as re-evaluation of various intensities of flavor defects of milk and ice cream seemed necessary due to marked changes in the flavor value on those score cards, and whereas a panel of judges was selected (1) to indicate those evaluations, and since the above mentioned judges were included in the panel, it seemed desirable to make a comparative study of the scores of the official judge and those of the remaining selected judges of the panel. Consequently, the average of the evaluations given by the judges other than the official for the various flavor defects of *distinct* intensity were plotted against those of the official. The data are shown graphically in figures 1 to 4, inclusive.

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* Deceased, February 9, 1942.

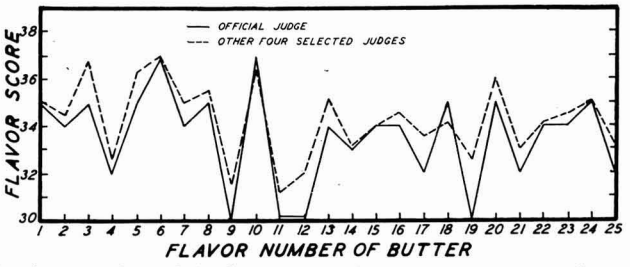

FIG. 1. A comparison of the flavor scores of butter having specific flavor defects of distinct intensity as given by the official judge and by other members of a selected panel.

In studying these charts it must be borne in mind that the actual effective range of flavor judging is wider than shown on the graph, for the values plotted on them are for flavor defects of *distinct* intensity only. The normal range of flavor scores including "without criticism" extends on the average about two points above the highest points shown on the graph. Furthermore, if the defects were *strong* rather than, *distinct,* the lower limit of the range would be somewhat lower for each flavor defect.

The identification of the flavors evaluated in figures 1 to 4 inclusive is made in table 1.

Data of these graphs show the relative agreement of the official judge and the remaining members of the panel, the flavors on which there is marked disagreement of evaluation, and the operative range of scores for the product.

Influence of the scoring range upon the reliability of scoring. With the exception of milk, the normal operative scoring range as designated by the judges on the panel (1) was relatively narrow, being 7 points for butter, 5.6 for ice cream and 3.4 for cheese. The operative range in milk flavor defects was 13.6, which was 1.94 times that for butter, 2.43 times that for ice cream, and 4 times that for cheese. If the average deviation in score

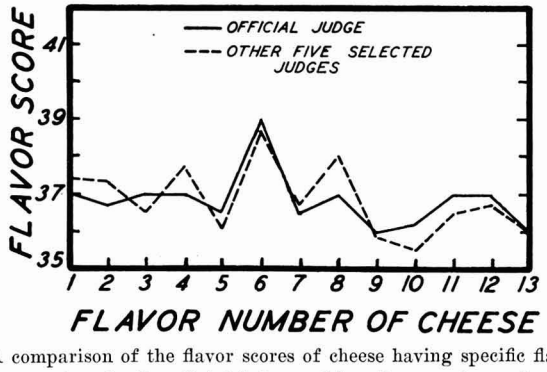

FIG. 2. A comparison of the flavor scores of cheese having specific flavor defects of distinct intensity as given by the official judge and by other members of a selected panel.

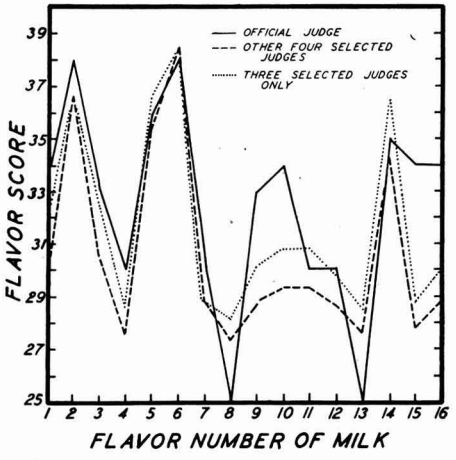

FIG. 3. A comparison of the flavor scores of milk having specific flavor defects of distinct intensity as given by the official judge and by other members of a selected panel.

between that of the official and the average of the remaining members of the panel were multiplied by those factors then it would appear that, considering the range of scores, the judging for ice cream and butter was more nearly standardized than that of milk and cheese. These data are given in table 2.

These data are very enlightening for they point out clearly the influence of the narrowness of the operative range of scoring on the resulting deviation in scores. This does not seem to have been fully appreciated heretofore, possibly giving rise to a false belief that little deviation in scoring or "closeness of scores" was an indication of excellent scoring. Actually, when the

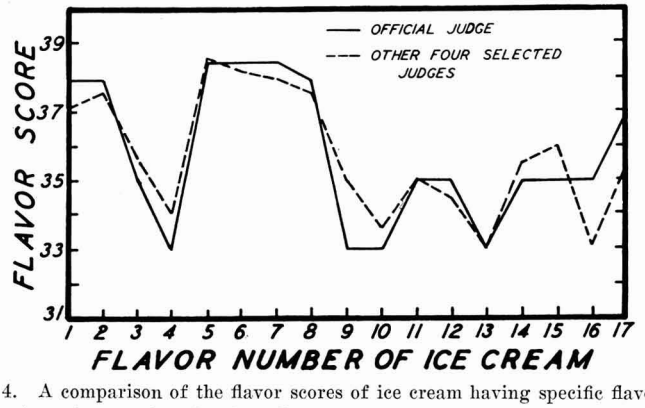

FIG. 4. A comparison of the flavor scores of ice cream having specific flavor defects of distinct intensity as given by the official judge and by other members of a selected panel.

TABLE 1

Identification of flavors in figures 1 to 4 inclusive

Flavor No.	Flavors corresponding to numbers on the graph for			
	Butter	Cheese	Milk	Ice cream
1	Acidy	Acidy	Bitter	Cooked
2	Bitter	Bitter	Cooked	Egg
3	Briny	Cowy	Cowy	Feed
4	Cheesy	Feed	Disinfectant	High acid
5	Coarse	Fermented	Feed	Lacks fine flavor
6	Cooked	Flat	Flat	Lacks flavor
7	Cowy	Fruity	Garlic	Lacks freshness
8	Feed	Heated	High Acid	Lacks sweetness
9	Fishy	Moldy	Malty	Metallic
10	Flat	Rancid	Metallic	Neutralizer
11	Garlic	Unclean	Musty	Old ingredient
12	Gasoline	Weedy	Oxidized	Oxidized
13	Malty	Yeasty	Rancid	Rancid
14	Metallic		Salty	Salty
15	Musty		Unclean	Storage
16	Neutralizer		Weedy	Unclean
17	Oily			Unnatural
18	Old cream			
19	Rancid			
20	Storage			
21	Tallowy			
22	Unclean			
23	Weedy			
24	Woody			
25	Yeasty			

operative range was relatively narrow in a product even poor judges might make a good showing in that product, whereas a relatively good judge might appear, on the basis of closeness of scores, to be doing poor judging if the operative range of scores were wide.

In this discussion it is fully appreciated that the values given are based upon an abstract defect designated on paper as having a certain intensity; that in actual judging the defect must first be noted organoleptically before a value may be placed on it; and that therein lies a most important factor in proficiency in judging which here is disregarded entirely.

TABLE 2

The average deviation between the official evaluation and those of the remaining members of the selected panel

Product	Average deviation per defect	Operative range of scores for flavor defects	Factor* used to standardize operative range to that of milk	Average deviation when multiplied by factor
Butter	0.98	7.0	1.94	1.90
Cheese	0.73	3.4	4.00	2.92
Milk	2.53	13.6	1.00	2.53
Ice cream	0.72	5.6	2.43	1.75

* Obtained by dividing operative range of milk by those of butter, cheese, and ice cream.

Inasmuch as the operative scoring range varied with the products, the number of off-flavors on which there was wide disagreement of evaluation might be expected to vary also. These data are included in table 3.

The percentage of defects in which the evaluations, as designated by the official judge and by the remaining members of the panel, deviated by more than two points was greatest in milk and least in cheese and were approximately the same for butter and ice cream. This might be expected considering the operative range.

Probably of more interest, however, were the specific off-flavors concerning which the judges exhibited wide disagreement of evaluation. The flavor numbers may be noted in the figures and identified in table 1. The off-flavors on which there was comparatively wide disagreement in butter were: "briny," "fishy," "garlic," "gasoline," "oily," and "rancid"; in cheese,

TABLE 3

Comparison of percentage of flavor defects of butter, cheese, milk, and ice cream on which the judges exhibited various deviations upon scoring

Product	No. of flavor defects	Av. deviation of panel members other than official from official score	Flavors in which evaluations deviated by	
			0 to 2.0 points	2.1 + points
			per cent	*per cent*
Butter	25	0.98	68.0	32.0
Cheese	13	0.73	76.9	23.1
Milk	16	2.53	12.5	87.5
Ice cream	17	0.72	70.6	29.4

none; in milk, "bitter," "disinfectant," "high acid," "malty," "metallic," and "rancid"; and in ice cream, "metallic," "unclean," and "unnatural."

As a general rule the members of the panel of judges agreed very well on the evaluations placed on the flavor defects. However, there were some exceptions, notably in milk judging, in which one judge tended to cut the defect more drastically than the other members. The influence of these cuts may be seen in figure 3 in which in one case the evaluations of the four panel members other than the official are considered in computing the averages and in another case in which the evaluations of three members only are considered.

CONCLUSIONS

The deviation in scores between a judge and the official without considering the operative range may not be a good criterion by which to measure the extent of standardization of judging among products.

With few exceptions a selected panel of trained judges may evaluate designated flavor defects of butter, cheese, milk, and ice cream comparatively close to those of an official judge and vice versa. Considering the

operative range of scoring, evaluations for flavor defects current to butter and to ice cream apparently are a little more standardized than those for milk and for cheese.

REFERENCE

(1) TROUT, G. M., *Ch.*, DOWNS, P. A., MACK, M. J., FOUTS, E. L., AND BABCOCK, C. J. The Evaluation of Flavor Defects of Butter, Cheese, Milk, and Ice Cream as Designated by Dairy Products Judges. JOUR. DAIRY SCI., **25**, No. 7: 557. 1942.

RAPID METHODS FOR ESTIMATING THE NUMBER OF SPERMATOZOA IN BULL SEMEN

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The concentration of spermatozoa in bull semen is exceedingly variable, both from ejaculate to ejaculate of the same bull and from bull to bull. Reliable estimates of the number of spermatozoa present in semen are of value in arriving at an indication of its quality, and, often are useful for research purposes, or for determining the rate at which semen should be diluted for routine artificial insemination. For maximum usefulness the estimate should not only be accurate, but should require a minimum of time.

The usual method of estimating the concentration of spermatozoa employs the standard equipment used in the counting of red blood cells. This method is time-consuming and requires considerable patience and skill.

Recently, Comstock and Green (3) have shown that the number of spermatozoa in ram semen can be accurately estimated by diluting the samples at a standard rate and measuring the relative light transmission in a photelometer. Burbank (2) for the guinea pig, Comstock and Green (3) for the ram, and Paršutin and Rumjanceva (5) for the stallion, have used rapid methods for estimating the concentration of spermatozoa in which diluted semen samples are visually compared with opacity standards. The first two papers cited deal with artificial standards in which barium sulfate is used to produce the opacity. Paršutin and Rumjanceva (5) used suspensions of stallion spermatozoa for their opacity tubes.

It is the purpose of this paper to report the results obtained when these rapid methods were used for estimating the number of spermatozoa in the semen of bulls and to present simplified directions for field application.

EXPERIMENTAL

Accuracy of counting with the standard hemocytometer method. There is no known method by which all the spermatozoa present in a semen sample may be counted. Consequently, the problem involved is one of sampling.

In the case at hand we are interested in obtaining from the entire ejaculate a sample sufficiently small in size and containing a sufficiently small number of spermatozoa so that by the methods available we can count with precision the spermatozoa present. If it is possible to obtain such a sample, and one which is representative of the ejaculate as a whole, it should then be possible to make reliable estimates of the number of spermatozoa in the ejaculate.

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When the hemocytometer method is used a small sample of semen is taken from the entire ejaculate by means of a standard red-cell dilution pipette. Bull semen normally contains so many spermatozoa that each sample must be diluted and further subsampled before estimating the number of spermatozoa present. The semen is usually diluted with a weak solution of Chlorazene, or other diluent, in the dilution pipette. The pipette is thoroughly shaken to insure proper mixing of the contents and two subsamples are obtained by introducing a small portion of the diluted sample into each of the two counting chambers of the hemocytometer. Actually, there is a further sampling of the particular subsample in question, for not all of the spermatozoa in each counting chamber are counted. In fact, each counting chamber is marked off into 25 large squares. These large squares represent but a small portion of the volume of the subsample in the counting chamber. In addition, in practice we have counted only 15 of the large squares in each counting chamber.

The counts of the spermatozoa present in a prescribed number of large squares serve as the basis for estimating the concentration of spermatozoa present in the ejaculate. The accuracy of the subsampling process may be determined by the degree to which the counts in the duplicating chambers agree. This agreement is spoken of as the *repeatability* of the estimate and is expressed mathematically by the correlation coefficient between one series of estimates obtained from the count of one chamber (I) on the counting slide and another series of estimates for the same samples obtained from the count of the second chamber (II) on the counting slide.

As has been stated above, we have followed the practice of counting the number of spermatozoa in 15 large squares in each chamber of the counting slide. For 65 ejaculates the data were recorded separately for each series of 5 squares counted. From these data it is possible to arrive at an estimate of the repeatability of the counts made and to determine whether or not it is necessary to make duplicate subsamples of each original sample, and whether or not it is necessary to count so many squares. The several correlation coefficients (r) calculated from these data are presented below:

r between count of first 5 squares of chamber I and total of 15 squares each for chambers I and II = 0.9873
r between count of first 5 squares of chamber I and second 5 squares of chamber I = 0.9692
r between count of first 5 squares of chamber I and first 5 squares of chamber II = 0.9679
r between count of first 10 squares of chamber I and total of 15 squares each for chambers I and II = 0.9959
r between count of 15 squares of chamber I and 15 squares of chamber II = 0.9910
r between count of 15 squares of chamber I and total of 15 squares each for chambers I and II = 0.9978

Since a correlation coefficient of 1.0 would indicate absolute agreement between counts of the two series of subsamples, these coefficients show that reliable estimates of the number of spermatozoa in the samples of semen taken from entire ejaculates by means of a small dilution pipette may be made by counting the number of spermatozoa found in 15 large squares of the two counting chambers of the hemocytometer slide. In addition, they indicate that fairly accurate estimates of concentration can be made by counting the spermatozoa in only 5 squares of one chamber, for the regression of the count of the first 5 cells on the total count of 15 squares each for chambers I and II accounted for 97.5 per cent of the variance between samples. However, these data do not give any indication of the repeatability of sampling from the entire ejaculate by the method used, for but one original sample was taken from each ejaculate and was then used for two subsamples on which counts were made. The question of the error of sampling from the ejaculate was left for consideration when more than one sample was taken from each ejaculate for estimation of spermatozoan concentration by the methods discussed below.

Comparison of hemocytometer count with density of diluted semen as determined by a photoelectric colorimeter. A photoelectric colorimeter constructed according to the design of Hand and Sharp (4) was used in these studies. A red Corning glass filter No. 241 was employed. For determinations of turbidity 1.0 ml. of an M/15 solution of crystalline sodium citrate (47.615 grams of $2\,Na_3C_6H_5O_7 \cdot 11\,H_2O$ per 1,000 ml. water distilled over glass) in a standard tube of 10.0 mm. diameter was placed in the colorimeter and the indicator brought to 100. A one-tenth ml. sample of the ejaculate was then added to the tube containing the citrate solution. The tube was then thoroughly shaken and a reading taken in the colorimeter. It was found that a straight line relationship existed between the Log $\frac{100}{\text{reading of sample}}$ and the mean of duplicate hemocytometer counts of another sample from the same ejaculate. No significant curvilinearity was demonstrated.

For 63 ejaculates the correlation between Log $\frac{100}{\text{reading of sample}}$ and the hemocytometer count was 0.9820. Thus a total of 96.4 per cent of the variance between samples was accounted for by the regression of the spermatozoan count on the Log $\frac{100}{\text{reading of sample}}$ as determined by appropriate calculations from the colorimeter readings. The regression equation for the observed relationship was: $E = 1816.4X - 68.5$; where $X = \text{Log}\frac{100}{\text{reading of sample}}$. The standard error of estimate was 8.8 per 0.0001 mm.^{3}, or 88,000 spermatozoa per mm.^{3} Using the mean count of 1,038,250 spermatozoa per mm.^{3} for the ejaculates studied this standard error of estimate of 88,000 spermatozoa per

mm.^{3} represents a variability of about 8.5 per cent of the mean count. The error of sampling ejaculates and of subsampling are both included in the standard error. Because of this small combined error, it is concluded that the number of spermatozoa per unit volume of ejaculate can be estimated by measuring the relative transmission of a standard beam of light through a diluted sample. The accuracy is comparable to that obtained by direct counting in the hemocytometer chamber. These data have served as a basis for preparing a calibration chart from which the spermatozoan equivalent for each reading of the colorimeter may be ascertained at a glance.

In an earlier investigation with a different colorimeter one of us found that three ejaculates from one bull did not fall on the same regression line as did eleven other ejaculates from three different bulls. The ejaculates from the one bull were found to contain an abnormally high proportion of leucocytes and other cellular debris. Ordinarily a trained observer would notice such samples in routine observation of motility and would use the method with due care.

Comparisons between hemocytometer count, density of diluted semen as determined in a photoelectric colorimeter, and visual determination of opacity rank. The two methods discussed above are not readily adaptable to field conditions. The hemocytometer method requires so much time that in one large unit where semen was collected from three or four bulls daily, counts on no more than two-thirds of the total number of ejaculates collected during a year were obtained even though a serious effort was made to count all ejaculates. A satisfactory photoelectric colorimeter is often too expensive for most farmer-owned artificial breeding cooperatives.

To determine the feasibility of using opacity standards with which to compare the density of bull semen diluted at a standard rate we have prepared barium sulfate standards after the recommendations of Peskett (6). Certain modifications have been incorporated to produce permanent standards with which semen diluted at the rate of 1 : 10 with an M/15 solution of crystalline sodium citrate might be compared.

The method of preparation of the standards is as follows: In order to prevent the growth of bacteria and molds one ml. of a saturated solution of mercuric chloride is placed in enough 100 ml. volumetric flasks to provide the necessary number of standards. One ml. of concentrated HCl is then added to each flask. Varying quantities of an M/40 solution of potassium sulfate are then added, the amounts depending upon the density required. The amounts required in our work have varied from 2.5 ml. to 60.0 ml. An M/4 barium chloride solution is added drop by drop at the rate of about 2.5 to 5.0 ml. per minute in sufficient amounts to completely precipitate all of the sulfate. As a margin of safety we have added an excess of nearly 100 per cent of the barium chloride theoretically required to precipitate the sulfate.

After standing 5 minutes at room temperature the flask is shaken thor-

oughly and placed in a 50° C. water bath. After the flask and its contents have come to the temperature of the bath, 20 ml. of warm filtered 3 per cent agar solution are added and the flask filled to the mark with warm distilled water. The agar solution is added to insure satisfactory distribution of the barium sulfate crystals in the suspension, thus making a permanent standard which does not require shaking before use. Gelatin may be used for this purpose, but it does not remain solid at high environmental temperatures.

After thorough shaking a portion of the mixture is placed in one of the standard test tubes of uniform 10.0 mm. diameter and uniform thickness. While the 100-ml. volumetric flask is kept in the water bath for future use the test tube is stoppered, quickly cooled by plunging it into ice water to set the agar immediately, and placed in the colorimeter where a reading is taken. If the tube is of the proper density as indicated by the colorimeter reading it may be used for a standard. If, however, it is of greater density than desired, the density may be decreased by judicious addition of warm

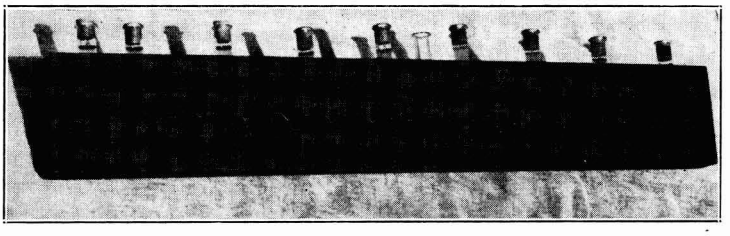

FIG. 1. Comparator used for purpose of comparing density of unknowns with opacity standards.

distilled water to the 100-ml. flask and the process repeated until standards of the proper density are obtained. Though this trial and error method of calibration appears cumbersome, it is not difficult when a suitable colorimeter is available for the density determinations. Care should be taken that the agar solution is not so greatly diluted that it fails to set. In addition, the material should be cooled quickly after it is placed in the standard tubes to prevent the precipitate from settling or flocculating, which makes comparison with diluted semen difficult. Following this procedure we have produced standards which are strikingly similar to diluted semen in appearance.

Table 1 gives the calculated spermatozoan equivalent for the Log $\frac{100}{\text{reading of standard}}$ of each standard opacity tube we used. The spermatozoan equivalent was calculated from the regression equation $E = 2059.7\ X - 281.9$ where $X = \text{Log}\ \frac{100}{\text{reading of standard}}$. The data in this table should serve as a basis for determining the density range of standards.

After the standard tubes were prepared they were placed in the simple

comparator illustrated in figure 1. The standards were placed in alternate positions in the comparator so that an unknown might be placed between each successive pair of standards. The comparator was then held up to a standard artificial light or a window, and the unknown moved about until it was in a position where the density of the unknown and a standard was closest. We designate the unknowns by the tube with which they most closely compare or half way between two standards if such a classification seems appropriate.

After such a comparator had been on trial in our laboratory for sufficient time to indicate that it could be used with success, we placed one in the hands of Mr. Maurice Johnson, technician of the New York Artificial Breeders' Cooperative at Syracuse, N. Y. We wished to determine whether or not our calibration of the standards with the colorimeter had been successful to the point where it was applicable to field operations, and to determine if it would be more accurate to calibrate the standards from actual hemocytometer counts of the semen obtained under field conditions.

In order to test these propositions we carried out the following procedure:

1. A one-tenth-ml. sample of each of 43 ejaculates was pipetted into a standard test tube containing 1.0 ml. of the sodium citrate solution.

2. After proper shaking, Johnson placed these diluted samples in the comparator and recorded the number of the standard tube with which each sample most closely compared. One of us followed the same procedure with the same samples. The classifications by the two operators coincided in every case.

3. These same samples of the 43 ejaculates were then placed in the photoelectric colorimeter, the readings taken, and the Log $\frac{100}{\text{reading of sample}}$ calculated.

4. Finally, a second sample from each of the 43 ejaculates was taken by means of the small dilution pipettes. These samples were then diluted and from each, two subsamples were placed in the chambers of a hemocytometer. Fifteen representative large squares were counted in each subsample.

The data obtained made possible the study of several different aspects of the problem at the same time. First of all, the question arose as to whether or not the human eye, in comparing an unknown diluted semen sample with a standard tube in a comparator, would place the sample closest to a standard tube, the density of which, as determined by the colorimeter, was most closely comparable to the unknown or whether there was a great difference between them. This question was studied by determining the correlation between the density for the standard (Log $\frac{100}{\text{reading of standard}}$) which most closely corresponded by visual comparison with each of the 43 semen samples, and

the actual density of each sample determined in the colorimeter (Log $\frac{100}{\text{reading of sample}}$). The correlation coefficient was 0.9694. This high correlation indicates that about 94 per cent of the variance is accounted for by the regression of actual density of samples on visual determination of density by comparison of the unknown with standards. From this fact we believe that the density standards are nearly identical with diluted semen in appearance both as measured by eye and by the colorimeter.

Secondly, we wished to determine the accuracy of calibration of the standard tubes by means of the colorimeter. This was done by determining the correlation between the Log $\frac{100}{\text{reading of standard}}$ for each standard with which each of the 43 samples of semen most closely compared, and the estimate of the spermatozoan concentration made on other samples from the same ejaculates with the hemocytometer. The calculated correlation coefficient was 0.9492. The standard error of regression, including both the errors of sampling and subsampling, was 15.7 spermatozoa per 0.0001 mm.^{3} or 157,000 spermatozoa per mm.^{3}

Finally, as a matter of interest we listed each of the 43 samples of semen by the number of the standard tube with which they compared. When a sample was judged to be between two standards and not more closely comparable to either one it was given the lowest number plus one-half, so that the numbers assigned, which we called opacity rank, were 1.0, 1.5, 2.0, 2.5, etc., up to 10.0. The correlation between the opacity rank and the hemocytometer count was 0.9598. This correlation coefficient may be interpreted as showing that 92 per cent of the variation of spermatozoan count of the 43 ejaculates was associated with changes in opacity rank. The standard error of estimate by this method was 14.0 spermatozoa per 0.0001 mm.^{3} or 140,000 per mm.^{3} This standard error, also, contains the errors of sampling and of subsampling.

On casual examination of the two standard errors of estimate one might assume that simply ranking the samples on the basis of opacity rank was a more accurate method than using the Log $\frac{100}{\text{reading of standard}}$ for each standard tube with which each of the 43 samples most closely compared. However, the difference in magnitude of the two standard errors was not significant when studied by Hotelling's method, recently discussed by Baten (1). This fact indicates that the difference in magnitude of the standard errors may be attributed to chance deviation.

Our studies indicate that opacity standards may be prepared by the methods discussed, calibrated in a photoelectric colorimeter, after the colorimeter has itself been calibrated for the particular filter and size of tubes used, and employed with only slightly less accuracy for determination

of spermatozoan concentration in bull semen than can be done with the hemocytometer.

Table 1 shows the essential information obtained when the standard opacity tubes were used.

TABLE 1

Spermatozoan equivalent for comparator determinations of relative density based on regression of count on opacity rank, and on regression of count on $Log \frac{100}{reading\ of\ standard}$

Standard tube number (opacity rank)	Spermatozoan equivalent (1,000's per mm.^{3})	$Log \frac{100}{\text{reading of standard}}$	Spermatozoan equivalent (1,000's per mm.^{3})
1.0	.....	0.1051	.....
1.5	122	0.1727*	74
2.0	240	0.2403	213
2.5	357	0.2888*	313
3.0	475	0.3372	413
3.5	592	0.4229*	589
4.0	710	0.5086	766
4.5	827	0.5599*	871
5.0	945	0.6108	976
5.5	1,062	0.6604*	1,078
6.0	1,180	0.7100	1,180
6.5	1,297	0.7669*	1,278
7.0	1,415	0.8239	1,415
7.5	1,532	0.8816*	1,534
8.0	1,650	0.9393	1,653
8.5	1,767	0.9697*	1,715
9.0	1,885	1.0000	1,778
9.5	2,002	1.0484*	1,878
10.0	2,120	1.0969	1,977

* Calculated values.

DISCUSSION OF RESULTS

Until the artificial insemination of dairy cows on a wide commercial basis became an established practice in the United States the semen of a bull was seldom examined unless he failed to settle the cows which he bred. It is also true that up until the time that the artificial vagina came into wide general use for the collection of semen, normal samples of semen were seldom seen. Thus, technicians in artificial insemination units, who were responsible for the collection and the handling of semen, were forced to gather most of their knowledge regarding its quality through the slow school of experience.

We have found the concentration of spermatozoa in bull semen to be an important factor in determining the extent to which the semen may be diluted for insemination. The more concentrated semen may be diluted to a much greater extent than can the less concentrated semen. In fact, in cooperation with the New York Artificial Breeders' Cooperative, we are diluting all semen for field use with the yolk-citrate diluter (7) at a rate determined by the count. Semen has been diluted at a rate varying from 1:2, for the less concentrated samples, to 1:14 for the more concentrated

samples, and our field results to date indicate that comparable rates of conception have been obtained. These data will be published later.

As pointed out previously, counting by means of the hemocytometer required so much time that it was not only impossible to count all of the ejaculates obtained each day, but it was not possible to apply any of the tests proposed as indications of quality of semen. Since satisfactory counts may be made by means of the visual determination of opacity rank, it is now possible to greatly shorten the time required to estimate the relative concentration of each ejaculate and to calculate the rate at which it should be diluted. Counts may be made on four ejaculates in five minutes or less. Thus, in the time saved the technician can set aside a small sample of diluted semen and run certain tests for quality which may be correlated with fertility data from the field.

It is suggested that many artificial insemination units now in operation might, by shortening the methods now used for determining the concentration of the semen produced by their bulls, save valuable time. Or if they are not now determining the concentration of the semen produced by their bulls, they may do so with this rapid method and use the semen more efficiently.

SUMMARY

The number of spermatozoa in bull semen may be estimated by means of the hemocytometer, the photoelectric colorimeter, and the visual comparison with opacity standards. The hemocytometer served as the basis of comparison for the other methods. It was shown that the concentration was determined in the colorimeter with an accuracy equal to that obtained with the hemocytometer.

The visual comparison of diluted semen with opacity standards resulted in an estimate of concentration only slightly less accurate than that obtained with the other two methods.

Directions are given for preparing opacity standards and for calibrating the standard tubes.

REFERENCES

(1) BATEN, W. D. How to Determine Which of Two Variables Is Better for Predicting a Third Variable. Jour. Amer. Soc. Agron., **33**: 695–699. 1941.

(2) BURBANK, R. C. The Quantitative Standardization of Sperm Suspensions by Means of Opacity. Quart. Jour. Expt. Physiol., **25**: 393–397. 1935.

(3) COMSTOCK, R. E., AND GREEN, W. W. Methods for Semen Evaluation. I. Density, Respiration, Glycolysis of Semen. Amer. Soc. Anim. Prod. Proc., **1939**: 213–216. 1939.

(4) HAND, D. B., AND SHARP, P. F. Rapid Photoelectric Determination of Carotenoid in Milk Fat. JOUR. DAIRY SCI., **22**: 729–735. 1939.

(5) PARŠUTIN, G., AND RUMJANCEVA, E. Standards for Determining Density of Stallion Sperm (Trans. Title). Konevodstov., **12**: 44–45. 1938. Citation from Anim. Breeding Abs., **9**: 206. 1941.

(6) PESKETT, G. L. Studies on the Growth of Yeast. IV. A Nephelometric Method for Counting Yeast Suspensions. Biochem. Jour., **21**: 460–466. 1927.

(7) SALISBURY, G. W., FULLER, H. K., AND WILLETT, E. L. Preservation of Bovine Spermatozoa in Yolk-Citrate Diluent and Field Results from Its Use. JOUR. DAIRY SCI., **24**: 905–910. 1941.

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ABSTRACTS OF LITERATURE

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CONTENTS

THE SCIENCE PRESS PRINTING COMPANY LANCASTER, PA.

PUBLICATIONS AND ABSTRACTORS

EDITORS

Dahle, C. D., Dahlberg, A. C., Elliker, P. R., Petersen, W. E., Tracy, P. H. and Weckel, K. G.

ABSTRACTORS

Archibald, J. G.
Babcock, C. J.
Bennett, F. W.
Berggren, Ruth E.
Brueckner, H. J.
Burgwald, L. H.
Bushnell, L. D.
Cole, W. C.
Cone, J. F.
Coulter, S. T.
Doan, F. J.
Dorsey, L. M.
Downs, P. A.
Ely, Fordyce
Erb, J. H.
Espe, D. L.
Frazier, W. C.
Fuller, S. A.
Garrett, O. F.
Glick, D. P.
Goss, E. F.
Horrall, B. E.
Huffman, C. F.
Irvine, O. R.
Josephson, D. V.
Lucas, P. S.
Lush, J. L.
Marquardt, J. C.
Martin, W. H.
Mueller, W. S.
Price, W. V.
Reece, Ralph P.
Sheuring, J. J.
Stebnitz, V. C.
Thomsen, L. C.
Trout, G. M.
Webb, B. H.
Weckel, K. G.
Weiser, H. H.
White, G. C.
Yale, M. W.

JOURNALS

American Butter Review
American Milk Review
American Journal of Diseases of Children
American Journal of Physiology
American Journal of Public Health
Archives of Pediatrics
Australian Journal of the Council for Scientific and Industrial Research

Biochemical Journal

Canadian Dairy and Ice Cream Journal
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Certified Milk
Cornell Veterinarian

Dairy Industries
Dairy World

Endocrinology

Food Industries
Food Manufacture
Food Research

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

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

Lancet
Le Lait

Milk Dealer
Milk Industry
Milk Plant Monthly

National Butter and Cheese Journal
New Zealand Journal of Science and Technology

Oil and Soap

Pacific Dairy Review
Proceedings of Society of Experimental Biology and Medicine

Refrigerating Engineering

Scientific Agriculture
Southern Dairy Products Journal

SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland

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ABSTRACTS OF LITERATURE

BOOK REVIEWS

1. **Food Manufacturing.** S. BLUMENTHAL. Chemical Publishing Company, Brooklyn, N. Y. 14 chapters, appendix of tables and information, index. 643 pages. $7.50.

A compilation of recipes, formulas, general review of materials used in foods, and food manufacturing processes. In the light of the title of the book, and the brief and often inadequate résumé on materials and methods, the scope of subject selected by the author seems too broad. With the exception of that given in a few chapters, the material does not provide new insight to the manufacture of foods. Unfortunately, the subject material as presented indicates unpardonable lack of organization and editing. Subheadings are indistinguishable from subject headings. Discussion is frequently unrelated to the heading. There are several oversights in arrangement of material derived from other sources. A list of edible fats and oils is duplicated on adjoining pages.

Statements picked from the text include, "The milk collected for the evaporated product (evaporated milk) must be even fresher and cleaner than that delivered in city markets"; "Milk sugar . . . does not have a sweet taste"; "The cold milk (evaporated) then passes through a homogenizer"; "Butter is the fat extracted from milk." The manner of presentation of the chapter, Dairy Products, Cheese, is not convincing, and indicates unfamiliarity with the industry and importance of its products. In it, Neufchatel, Bakers, Roquefort, and Iowa Blue cheese, acidophilus and kefir milk are discussed at length. American type and processed cheese, butter manufacture, beverage milks are each discussed briefly. Powdered milk and other important products are not discussed. A few formulas (household type) for ice cream are mentioned in a section on Candies. The standards cited for evaporated milk are not up to date. A taste and glass test (fermentation and sediment) are listed as methods for appraising the quality of milk powder. Several chapters contain lists of types of products used in foods, but frequently sources, characteristics, qualities or properties cited for one are not cited for another. Chapters which may be of interest to manufacturers of dairy products are: Cocoa, Chocolate Products; Nut Products (specific problems in their use not mentioned), Fountain Supplies, Food Colors. Other chapters include Basic Food Ingredients; Confectionary, Coffee, Tea; Gelatin Desserts, Pudding Powders; Sauces, Condiments, etc.; Fish, Fried Foods. Nut Products; Pie Fillings; Specialty Foods, Bakers Products; Fruits, Vegetables, Fruit Juices; Canned, Dried Fruits; Jellies, Preserves. Citation to references is not made in the text; a brief list of books and bulletins is cited in the appendix. K.G.W.

2. **Food Industries Manual, 10th Edition.** Leonard Hill, Ltd., 17 Stratford Place, London W. 1, Publisher. Chemical Publishing Co., Inc., 234 King St., Brooklyn, New York, Distributor.

This manual on food ingredients, processes, tests is from the publishers of the British trade publication "Food Manufacture." It contains 126 pages of advertisement of food products and manufacturing equipment, an index of manufacturers and their addresses, trade marks and names. It contains 232 pages divided into 8 sections as follows: Wheat, Milling, Flour Baking, Flour Confectionary; Sugar Confectionary, Candy, Chocolate, Jams, Jellies; Canning and Preserving; Meat Products; Formula and Figures for the Dairy Industry; Packing, Packing Materials, Containers, Packing Machinery: Food Storage, Refrigeration, Insulation, Air Conditioning, Composition of Foods. Further, the book contains 75 pages of bibliography of technical books covering all phases of food manufacture, and available from the publisher. Each of the eight sections is in alphabetical encyclopaedia style, arranged by individuals familiar with the respective phases of food processing. Many of the descriptions or discussions have been selected, or condensed from issues of "Food Manufacture." The section on "Dairy Industry" is very brief, containing only 7 pages of formula, tables and tests. This section could be considerably improved, and contains nothing of recent origin, nor unavailable in most American sources of information. While many discussions are necessarily condensed they are presented in practical form and give insight to British methods. K.G.W.

3. **Introduction to Parasitology.** A. S. PEARSE. 1942. Charles C. Thomas, 220 E. Monroe St., Springfield, Ill. 334 pages, 448 illus. Price, $3.50.

"In this book an attempt is made to survey the field of parasitology briefly. It is hoped that it will be useful in 'looking up things,' whether it is used in a class room, on a farm or a fishing boat, in a packing house or a seaside laboratory. It is intended to accompany a course where teacher and student are attempting to gain first-hand information for themselves and think about the significance of such information."

Thus the author points out the purpose of a book that seems destined to fulfill that purpose. The text is replete with drawings of parasites and such parts and organs as will aid in identification. Diagrammatic representation of many life cycles helps the casual reader or the student to grasp the significance of the habits of those species important to man. Along with a short discussion of the relationships of parasites and hosts, nomenclature and ecology is a list of the phyla and classes in which parasites occur.

Parasites, from hexamita to mammalia, which infect man directly or infect species of the animal kingdom in which man is interested, are described, their life cycles, habits, pathogeneses and control discussed con-

cisely but completely enough so that the book "will be useful in 'looking up thing'" whether one be interested in parasite control or merely in satisfying a curiosity or in broadening his knowledge.

Considerable space is given to malaria and hookworm infection. One chapter is devoted to fecal examinations and identification of parasitic ova, spores and cysts. S.A.F.

BACTERIOLOGY

4. The Serological and Biological Classification of Hemolytic and Nonhemolytic Streptococci from Human Sources. LOWELL A. RANTZ, Stanford University, School of Med., San Francisco. Jour. Infect. Dis., *71,* No. 1: 61–68. July–Aug., 1942.

Serological classification by the method of Lancefield was carried out in 392 strains of hemolytic and nonhemolytic streptococci from human sources other than the respiratory tract. Biological classification was undertaken on the basis of tests for growth at 10° and 45° C., growth in the presence of 6.5% NaCl and growth in the presence of 0.1% methylene blue. Lancefield's groups A, B, D, F, G, and H were represented by 6.6, 8.6, 47.1, 9.5, 3.3, and 6.6% respectively of the 392 strains. Two strains were classified as group C. Seventy-one (18.1%) could not be identified with any of Lancefield's groups. Identification of group D strains could be made on the basis of the biological tests. Of the 185 strains in this group, 4 nonhemolytic strains and 1 hemolytic strain failed to grow in the methylene blue medium and 1 strain of each type failed to grow at 10° C. All other group D strains grew rapidly under all of the test conditions. The biological methods employed were of little value in differentiating members of the other Lancefield groups or the unclassified strains. J.F.C.

5. Further Experiments on Accessory Growth Factor Requirements of the Brucella Group. STEWART A. KOSER AND MARJORY H. WRIGHT, Univ. Chicago. Jour. Infect. Dis., *71,* No. 1: 86–88. July–Aug., 1942.

This is a continuation of studies reported earlier (JOUR. DAIRY SCI., *25,* No. 1: A6, Jan., 1942). Pure biotin and biotin methyl ester were compared with the previously employed biotin concentrate in their ability to promote the growth of 3 strains of *Brucella abortus.* Results were the same for all three lots of biotin. In each case 0.01 μg. of biotin per ml. of medium was used. In an experiment employing decreasing amounts of biotin it was shown that as little as 0.000003 μg. per ml. of medium consistently supported light growth of the test organisms. In another study in which pure pyrimidine and pure thiazole were tested for their ability to replace thiamin in the medium, it was found that thiazole had no growth-promoting effect on

the test organisms, but that pyrimidine could be substituted for thiamin in media for the seven brucella strains employed. J.F.C.

BUTTER

6. The Visual Mold Test Under Kansas Conditions. W. J. CAULFIELD, F. E. NELSON, AND W. H. MARTIN. Natl. Butter & Cheese Jour., *33*, No. 9: 28. Sept., 1942; *33*, No. 10: 36. Oct., 1942.

During 1940–41 eight Kansas creameries furnished 870 samples of cream which were tested for visual mold, acidity, yeast and mold and finally graded organoleptically. Excessive mold was not a serious defect, although most apparent in warmest months. Cream quality was similar in December, March and July but in May the percentage of first grade cream dropped from about 78 to 56%; feed and weed flavors were noticeable defects. Quality in terms of acidity was better during the cold months.

The visual mold test is a reasonably accurate means of determining the mold content of cream. It is not closely related to yeast content, nor to titratable acidity; it will not detect low grade cream so classified organoleptically by defects other than mold. The lack of satisfactory agreement between the visual mold test and other common tests for cream quality arise primarily because factors which determine mold content do not parallel those which cause other defects in cream. It would be a serious mistake for any creamery or cream station operator to use the mold test as the sole criterion of cream quality. W.V.P.

7. Studies on Storage Butters Showing Surface Deterioration. A. H. WHITE, Dom. Dept. Agr., Ottawa. Sci. Agr., *23*, No. 1: 41. 1942.

Bacteriological and chemical studies were made on 45 samples of storage butter, all but seven of which had declined in grade during storage as a result of the development of surface flavor defects. Tallowy, stale, unclean and fishy flavors were the chief types of defects. With the exception of two samples all butters had storage periods of one to 16 months at temperatures ranging from 15° to −20° F. They were gathered from a wide area and the majority were produced during the summer months. Sampling and examinations were such as to allow comparisons between the interior and surface characteristics of the butter. Bacteriological studies included total, proteolytic, lipolytic and oxidase-positive counts. In addition, colonies were picked into litmus milk tubes and the subsequent reaction determined. The pH, Cu content and peroxide number were determined, the latter determination being made on both interior and surface samples.

There did not appear to be any correlation between either total bacterial counts or the numbers of proteolytic, lipolytic or oxidase-positive organisms and specific flavor defects. However, in many samples the numbers, espe-

cially at the surface, were greatly in excess of what might be expected in well-pasteurized cream butter. Three samples had counts over 1,000,000 per ml. whereas many others had total counts well below 25,000 per ml. on both surface and interior butter. Counts of proteolytic, lipolytic and oxidase-positive types tended to be related to total counts.

Litmus milk tubes revealed that the majority of the organisms were acid non-coagulating or inert forms. The predominant morphological types in most samples were gram-positive cocci, although other types were regularly present.

The pH values ranged widely and 31% of the degraded samples were below pH 6.05. All samples having fishy flavors had low pH values, two of these being above the average in copper content. Peroxide values were not excessively high and could not be correlated to flavor defects.

It is pointed out that the defrosting period is one in which bacteriological and chemical changes might occur, in addition the period between churning and going into cold storage. An extensive list of references is included. O.R.I.

8. A Study of Methods of Accelerating the Swift Stability Test. V. C. MEHLENBACHER, Swift and Co., Chicago, Ill. Oil and Soap, *19*, No. 8: 137. 1942.

Although the Swift fat stability test is probably the most satisfactory test known for determining the stability of a fat toward oxidation the length of time required to obtain results makes it impractical for commercial use.

Attempts to accelerate the test by means of metal catalysts were not satisfactory as the results did not correlate those obtained by the original method. Satisfactory correlation with existent data was obtained when the tests were made at 110° C. with a saving of 60% of the time previously required to complete the test. The ratio of the time required at 97.7° C. to the time required at 110° C. was 2.5. V.C.S.

CHEESE

9. The Manufacture of Cornhusker Cheese. E. L. REICHART AND P. A. DOWNS. Nebr. Col. Agr. Bul. 342. June, 1942.

This cheese has been developed to satisfy the demand for a mild-flavored, soft-textured natural cheese. The equipment required corresponds to that used for making cheddar cheese.

The milk should be pasteurized, cooled to 86°–88° F. and 2½% starter added. Rennet is added at the rate of 4½ ounces per thousand pounds of milk. After the curd is firmer than for cheddar cheese, about 45 minutes setting, it is cut into ⅜-inch cubes. After 3 to 5 minutes stirring, it is heated to 98° in 45–60 minutes and held until somewhat less firm than cheddar

cheese curd but firmer than brick cheese curd. The curd is then covered with water at 65°–70°, stirred and drained. Salt is added at the rate of 3 pounds per 1000 pounds of milk and the curd stirred 15 minutes. The curd is then placed in hoops to make 5 pound cheeses and pressed. The entire process should require about 2 hours. The cheese is dressed and pressed overnight. It is coated with "dubbledipp" wax after two days. It may be cured at 65° and 70–80% relative humidity for 30–60 days or for 6–12 months at 45°. It can be stored for 1–2 years at 32°–35° without material change.

The cheese should contain 40–45% moisture. Its body is quite open but develops a smooth soft texture. The flavor is pleasingly mild. It slices well and does not crumble. A.C.D.

10. pH Control in Dairy Industries. Food Mfr., *17*, No. 8: 227. Aug., 1942.

The properties of milk depend among other things upon salt and pH. Salt ions are affected by the pH. Coagulation is determined by the hydrogen ion concentration. A definite relationship exists between the pH and the titratable acidity. One can be fairly well determined from the other. A different relationship exists in sour milk between pH and total acidity.

pH is important in the commercial preparation of casein, by the acid precipitation process and indicates better what milk to use for cheesemaking. The author gives uses of the pH. J.C.M.

11. Improved Practices Aid Cheese Makers. J. C. MARQUARDT. Food Indus., *14*, No. 6: 49–50. June, 1942.

Adequate sterilization of utensils and equipment, better santitation, mold control, fly elimination, and composition control have cut losses and improved quality in cheese manufacture. Other food processors can profit from what the cheese makers have learned.

Uses of chlorine to keep apparatus and laboratory sweet and also destroy micro-organisms are given. Rules on how to use chlorine are included. Fly control and methods of mold control and procedures for salt control in the cheese industry are given. J.C.M.

12. Directions for Making Cheese Spreads. C. R. BARKER. Food Indus., *14*, No. 6: 52–55. June, 1942.

Spreads can be made in almost unlimited quantities since aged cheese is not required. Profits are greater than for processed cheese. Raw material inventory is low and equipment not very costly. Formulas and processing procedure are given in this article. Very good information on cheese and cheese spreads is included for those having a direct interest in the subject. J.C.M.

13. The Volume and Composition of Dairy-Factory Drainage in Taranaki. P. A. VEALE, Taranaki Serv. Labs., Hawera, N. Z. New Zeal. Jour. Sci. and Technol., *23*, No. 3A: 166A–180A. 1941.

The volume of drainage from a large, non-pasteurizing factory was 0.26 gallons per pound of cheese and 0.84 gallons from a small, pasteurizing factory. A large butter-factory, making 1000 tons per year, had 0.71 gallons of drainage per pound of butter, while a smaller plant, making 250 tons per year, had 1.35 gallons of drainage per pound of butter. Lactic-casein factories ran high in quantity of drainage with 3.37 gallons per pound of anhydrous casein.

Figures are given to show the effect of time of day and of season on the volume of drainage and the drainage was analyzed for pH, titratable acidity, total solids, nitrogen, oxygen absorption and biological oxygen demand.

W.C.F.

14. Cracked Rinds in Cheddar Cheese. R. M. DOLBY, Dairy Res. Inst., N. Z. New Zeal. Jour. Sci. and Technol., *23*, No. 4A: 194A–201A. 1941.

To secure a close rind the pieces of curd on the surface of the cheese must be brought into intimate contact with each other while they are still warm, and any disturbance of the curd after it has cooled must be avoided. A porous layer (outer bandage) must be present between the cheese and the hoop to facilitate the escape of the whey, and the cheese must be well pressed. Wrinkles, holes, or loose patches in the bandage may produce cracks by causing uneven shrinkage of the rind during the drying-out of the cheese.

Variations in the composition of the curd have only minor effects on the rind. The moisture content of the curd at hooping could vary much without affecting the rinds. Low acidity may favor cracks. If the temperature of the curd is dropped to less than 70° F. or if curd from the previous day is added cracks are produced. If the curd is disturbed in the hoop, rind cracks may be produced. Scalding of the cheese surface helps avoid cracks.

W.C.F.

15. The Progress and Present Position of Research on Cheese Starters in New Zealand. H. R. WHITEHEAD AND G. J. E. HUNTER, Dairy Res. Inst., N. Z. New Zeal. Jour. Sci. and Technol., *23*, No. 1A: 40A–46A. 1941.

Work in New Zealand on cheese starter problems is reviewed with special reference to air-borne bacteriophage as the cause of failures of starter cultures. While the ultimate source of the phage is obscure, it was shown to be carried by milk or by whey from dust or from spray from the whey

separator. Use of a heavy inoculum in transfer of cultures helped suppress the phage. Other suggested remedies were: (a) To destroy the phage in the air. (b) To prepare the starter in the factory using special devices to exclude air-borne phage. (c) To prepare the starter outside the factory in a special building where the air is free from phage. W.C.F.

16. The Utilization of Whey. D. MURRAY SMILLIE AND JOHN WIGHT. Food Mfr., *17*, No. 6: 158–162. June, 1942.

Cheese whey is a highly nutritious product containing one half of the milk nutrients. Its composition is such that it should be used in the human dietary.

Much time has been spent in finding a method of processing whey and it has been found unsatisfactory to process it like other dairy products. Improvements are the aim.

Whey contains more than half the solids of whole milk and over 70 million gallons of milk are made into cheese each year. The problem is to find a product made of whey to put on the market and how food manufacturers can assist. The author gives composition of whey. Recent attempts to form a market have favored processing which conserved all the solids of the whey instead of separating it into lactose or albumen and other constituents.

Experiments have been carried on in England to consider the use of whey products for butter. These products have high food value. Albumen recovered commercially has been used for stock food and ground with lime for fertilizer. Its use as an egg white substitute has not been successful.

Whey cheese is discussed, also dried and condensed whey, and their process. Sweetened condensed whey has been experimented on by Webb, an American. It is made on the principle of sweetened condensed milk. The method of obtaining lactose from whey was discussed. A process developed by the West of Scotland Agricultural College gives whey a general appearance of milk, and it has been used in making ice cream. Candy makers and soda fountains have also been using it.

The salty flavor is hard to dispose, of; it has been suggested that whey be added to some cereals. It is also used in the manufacture of some cream soups. Many fruit mixtures can be made with whey. Much room for more improvement is emphasized. J.C.M.

DISEASE

17. On the Isolation and Growth of a Bacteria-Free Strain of Trichomonas Fetus in Minnesota. MORRIS D. SCHNEIDER, St. Paul, Minn. Jour. Amer. Vet. Med. Assn., *101*, No. 787: 245. Oct., 1942.

Using a thermostabile semi-solid medium and a Glaser-Coria "V" tube, isolation was made of a strain of *Trichomonas fetus* in Minnesota.

For bacteria-free isolation, it is recommended that the material be taken from a direct vaginal wash of an infected cow, immediately washing the trichomonads by centrifugation and concentrating them to a small volume of 1 to 2 cc. of liquid medium. The washing seems to increase the motility of the trichomonads, thereby making the chance of isolation more likely.

Cultivation on sodium citrate and sodium carbonate mediums has been carried on through 20 transfers. S.A.F.

18. **Prothrombin Activity During Pregnancy and Lactation.** J. B. FIELD, R. S. OVERMAN, AND C. A. BAUMANN, Dept. Biochem., Col. Agr., Univ. Wis., Madison. Amer. Jour. Physiol., *137,* No. 3: 509–514. 1942.

"The anticoagulant 3,3′-methylenebis (4-hydroxycoumarin), was found to be only 18% as effective on lactating rats as on non-lactating rats. The relation of hemorrhagic sweet clover disease in cattle to this finding is discussed." D.E.

19. **Studies on the Detection of Mastitis in New Zealand Dairy Herds. III. An Investigation into the Application of the Bromthymol Blue Test for Mastitis in the Field.** F. H. MCDOWALL, Dairy Res. Inst., N. Z., J. P. JAMES, Dept. of Agr., N. Z., AND A. H. WARD, N. Z. Dairy Board. New Zeal. Jour. Sci. and Technol., *23,* No. 4A: 223A–236A. 1941.

It was found that there was a lack of uniformity in the reading of the test by various workers, due to color blindness and other factors. It is recommended that the test be better standardized. A positive test is considered of more reliable significance than a negative one. The test picked out only about 40 to 50% of the samples with a high leucocyte count; but about 80% of the positive samples had a high leucocyte count. W.C.F.

20. **Studies on the Detection of Mastitis in New Zealand Dairy Herds. IV. An Examination of the Hopkirk Assessment and of the Breed Cell Count Method of Estimating the Leucocyte Content of Milk Samples.** J. P. JAMES, Dept. Agr., N. Z., AND F. H. MCDOWALL, Dairy Res. Inst., N. Z. New Zeal. Jour. Sci. and Technol., *23,* No. 4A: 237A–243A. 1941.

The Hopkirk method for the estimation of the leucocyte content of milk by the examination of a smear of gravity cream from the sample was found to give reasonably concordant results with the same or different workers in the same laboratory, but divergent results when workers were from different laboratories. When this method was compared with the Breed method, it was found that with a Breed cell count of one million per milliliter taken

arbitrarily as the boundary line between negative and positive mastitis infection, the Hopkirk classes 0 to 2 give a reasonably uniform indication of the negative cases and 4 to 6 of the positive cases.

The Breed method gave wide variations in results on duplicate samples examined by the same observer or by different observers. W.C.F.

FEEDS AND FEEDING

21. The Effect of Fineness of Grinding Grain on Milk Production. T. M. Olson. S. D. Agr. Expt. Sta. Bul. 358. Feb., 1942.

In two trials with eight cows in the early stages of lactation, coarsely ground grain ($\frac{3}{4}$-inch mesh) was more palatable than finely ground grain ($\frac{1}{16}$-inch mesh) and was found equal or superior to finely ground grain for milk production. The cost of grinding increased with increase in the degree of fineness of grinding. J.G.A.

22. Ascorbic Acid in Goats' Milk, Blood, and Tissues. Martha S. Richmond, C. D. Grinnells, and G. H. Satterfield. N. C. Agr. Expt. Sta. Tech. Bul. 68. May, 1942.

During a period of eighteen months, 430 milk samples and 260 blood samples were taken from five Toggenburg goats receiving a normal diet. The ascorbic acid content of these samples as determined by titration against sodium 2, 6-dichlorobenzenoneindophenol fell within the range of 0.5 to 2 mg. per 100 cc. for about 90% of the milk samples. Most of the blood samples contained between 0.4 and 0.8 mg. per 100 cc. No consistent relationship between the ascorbic acid content of the milk and that of the blood was observed.

Each goat was injected intraperitoneally three times with ascorbic acid (twice with 1 g. and once with 2 g.). Following the injections, a rapid, large rise in blood ascorbic acid, a rapid, very large rise in urine ascorbic acid, and a slower, small rise in milk ascorbic acid were observed.

Half of a group of twelve goats were fed a vitamin C-free diet; the ascorbic acid contents of the milk, the blood, and some tissues were determined.

The diet consisting of a grain ration only was deficient in other factors as well as in ascorbic acid and was not adequate for normal reproduction and lactation. The two goats already on the diet would not breed. The other four were placed on the diet during gestation; two aborted—one after two months, the other after three and one-half months; all four were subnormal in milk production.

After the goats were on the diet for two to three months, the blood ascorbic acid was about 0.4 mg%, or approximately $\frac{2}{3}$ the normal average concentration. There was also a decrease in milk production.

In the two non-milk goats which were not bred, the blood ascorbic-acid concentration decreased approximately $\frac{1}{3}$ in fifty-five days, and another $\frac{1}{3}$ in the next two hundred days on the diet.

Tissue analyses indicated that the decrease in blood ascorbic-acid concentration was accompanied by a similar decrease in liver ascorbic-acid concentration but not in that of the adrenal. Apparently, there was a decrease in total ascorbic acid of both the adrenal and the liver as a result of atrophy.

There was more deposited fat in the peritoneal cavities of the experimental animals than in that of a control animal, even though the experimental animals had apparently lost weight.

Estimations were made which show that the intake of ascorbic acid in a normal ration may be as much as the goat loses in milk, urine, and kids. Also, the amount excreted by goats on the vitamin C-free diet could have come from tissue stores.

The results raise the question as to the ability of the goat to synthesize ascorbic acid.

An extensive bibliography is appended. J.G.A.

23. A Comparison of Legume Hays for Milk Production. C. C. HAYDEN. Ohio Agr. Expt. Sta. Bul. 631. July, 1942.

This project was begun in 1925, in an attempt to answer the frequently raised question, whether alfalfa hay was sufficiently more valuable than clover hay to warrant the extra expense of seed cost and soil requirements necessary to grow it successfully.

Three groups of three Holstein heifers each, were fed ground shelled corn with alfalfa, soybean, and clover hay, respectively, until they had gone through 4 years or more of milk production. The growth of these heifers was normal until they came into milk. The live weights then lagged until at maturity they averaged 70 to 140 pounds below the average of half sisters kept on more varied rations. The reproduction of the groups on the different kinds of hay was about equal to the normal for the herd. Breeding and other difficulties while the cows were on these rations through four lactations did not seem to occur more often than among other cows kept in the same barn and on more normal rations. The birth weights of the calves carried while the cows were on these restricted rations were somewhat lower than those of their dams in the alfalfa and soybean groups and slightly higher in the clover group. The average weight of those from the clover group was the highest, owing in part to the large calves from one cow in the clover group. The consumption of feeds and production of milk were highest in the clover-corn group, but the difference was scarcely significant. In none of the groups was the consumption as high as was anticipated, owing, no doubt, to the restricted rations.

The results of this long-time test do not show a marked superiority of

alfalfa hay over clover or soybean hays for milk production. Clover protein seemed to be fully equal to alfalfa protein for milk production. If production was limited by the quality of the protein, the clover protein must have been the best. No single kind of legume roughage should be fed exclusively over long periods of time. J.G.A.

24. War Emergency Plans for Raising Calves and Heifers. W. E. KRAUSS. Ohio Agr. Expt. Sta. Bi-Monthly Bul. Sept.–Oct., 1942.

A discussion of the nutritive needs of calves and methods of rearing, with emphasis on a modified dry-feeding system for use in the war emergency, or whenever whole milk, skim milk and skim milk powder are too high-priced to be economical. A suggested schedule for emergency feeding is outlined. J.G.A.

25. Grass Silage in War-time. H. A. HERMAN AND A. C. RAGSDALE. Mo. Agr. Expt. Sta. Circ. 234. May, 1942.

Three methods of making grass silage under war-time conditions are outlined, (1) use of corn meal, or other cereal grains; (2) no preservative used, crop allowed to wilt to 65% moisture; (3) use of dry or green sugary crops such as sorgo or corn fodder. A home-made moisture tester for use with (2), developed by the U.S.D.A., is described and illustrated. Instructions are given for feeding grass silage. J.G.A.

26. The Relation of Grain Feeding to Milk Production. A. A. BORLAND, A. L. BEAM, AND P. D. JONES. Pa. Agr. Expt. Sta. Bul. 424. March, 1942.

Twenty-four Holstein and Brown Swiss cows were fed a maintenance ration of alfalfa hay and corn silage. Production was largely from grain, fed at 70, 80, 90, 100, 110, and 120% levels of Haecker's feeding standard. Cows were milked 3 times daily, and received daily exercise but no pasture. Each level of grain feeding was continued for two full lactation periods. Protein content of the grain mixture was approximately 14%. During a third year twenty-two of the cows were allowed all the grain they would eat.

Increasing the grain allowance of dairy cows up to the limit of their capacity to consume grain resulted in a great increase in milk production and under careful management was accompanied by no injurious results to the animals.

Decreasing the grain allowance to 70% of the amount required for milk production by the Haecker standard had no detrimental effects other than a lessened milk yield and a thinner condition of flesh.

The law of diminishing returns operated with increasing allowances of grain, since increments of grain at the higher feeding levels brought continually decreasing yields of milk per unit of grain.

The feed cost of milk production gradually increased with increased allowances of grain.

A normal allowance of grain according to the Haecker feeding standard was most profitable at the average prices of feeds and milk prevailing in Pennsylvania in March, 1942.

There was an upward trend in the value of milk over feed cost per cow at the higher levels of grain feeding when the price of milk was higher than the average for the state.

A convenient reference table is submitted as a guide to dairymen in feeding the most profitable allowance of grain per cow daily with varying relationships between the price of milk and the price of grain. J.G.A.

27. The Digestibility by Dairy Cows of a Grass Silage. P. D. SEARS AND F. B. SILL, Dept. Sci. Indus. Res., N. Z. New Zeal. Jour. Sci. and Technol., *23*, No. 1A: 50A–56A. 1941.

The pit silage was made mostly of perennial rye-grass and white clover, but contained some other grasses and some weeds. Molasses was added during filling at the rate of 30 pounds per ton of green material. The pit was covered with a layer of soil. The resulting silage was palatable and acid and did not appear to have been over-heated, but the animals apparently did not eat to capacity.

Tests on digestibility, made by feeding dairy cows, showed that breed, size and level of production of the cow had no influence on its ability to digest the silage. The figures for digestibilities of the nutrients were within the range of variation of figures of others for similar fodder. W.C.F.

28. The Relation of Plane of Nutrition to Milk Production and Milk Composition in New Zealand. I. Effect of Subnormal Feeding. W. RIDDET, I. L. CAMPBELL, F. H. MCDOWALL, AND G. A. COX, Dairy Res. Inst., N. Z. New Zeal. Jour. Sci. and Technol., *23*, No. 2A: 80A–98A. 1941.

It had been noted that the yield of cheese per pound of fat in milk showed variations which were independent of the normal variations due to lactational change in milk composition. In an effort to find a partial explanation of this phenomenon, 6 cows were fed in three groups with one group on a normal plane of nutrition throughout, and the other two groups on full and half production ration on the double-reversal system. It was found that: (a) Substitution of fresh pasture for part of the ration of meals and hay feed had no influence on the production or composition of the milk. (b) Change from a full to a half ration caused a reduction in yield, but less than was expected. (c) On the reduced ration there was no consistent change in the fat content of the milk, but there was a definite decrease of 0.3–0.5% of solids-not-fat in the milk. The solids-not-fat returned to nor-

mal on the change back to full rations. (d) The change to half ration was accompanied by a rise in the iodine value and the saponification value of the butterfat. Hence the seasonal decline in solids-not-fat of milk in spring and autumn may be due to subnormal nutrition. The response of the animals to normal feeding after underfeeding was found variable and was influenced by the stage of lactation. W.C.F.

29. The Relation of Plane of Nutrition to Milk Production and Milk Composition in New Zealand. II. Effect of Subnormal Feeding. W. RIDDET, I. L. CAMPBELL, F. H. MCDOWALL, AND G. A. COX, Dairy Res. Inst., N. Z. New Zeal. Jour. Sci. and Technol., *23*, No. 2A: 99A–112A. 1941.

Six cows, two groups of three, in a later stage of lactation than those in Part I, were used on the double reversal system. The results confirmed the observation that a subnormal plane of nutrition depresses milk yield and the solids-not-fat content of the milk and raises the iodine value of the butterfat. The solids-not-fat content of the milk may drop below the legal limit, even with cows in advanced lactation. There may be a small decrease in lactose in the milk, but the decrease in solids-not-fat is largely due to a fall in protein content. When cows are in mid-stage lactation or later, underfeeding is more likely to reduce total yield on return to full rations than when underfeeding had taken place earlier. W.C.F.

30. Reducing the Cost of Producing Dairy and Poultry Products in Missouri. H. FRAME. Mo. Agr. Expt. Sta. Circ. 237. June, 1942.

A discussion of ways and means of cutting production costs. Emphasis is placed on (1) providing needed buildings and equipment, (2) fuller utilization of present plant, (3) more advantageous use of labor, (4) more home grown feed, (5) higher quality stock and (6) intelligent planning. J.G.A.

FOOD VALUE OF DAIRY PRODUCTS

31. Ascorbic Acid Content of Cow's Milk After Five Years of Continuous Lactation. A. D. HOLMES, FRANCIS TRIPP, E. L. Patch Co., Boston, Mass., AND G. H. SATTERFIELD, Univ. N. C., Raleigh. Food Res., *7*, No. 5: 370. Sept.–Oct., 1942.

An eleven-year-old Holstein cow which failed to become pregnant during a five-year lactation period, but which continued to give milk, was maintained in a milking herd and her milk analyzed for ascorbic acid during the 62nd to 65th month of lactation. The ascorbic acid was determined at eight bi-monthly intervals, varying from 20.30 mg./l. to 33.54 mg./l. and averaging 24.91 mg. The milk flow was relatively constant, varying from 7.66 to

8.37 liters per day with an average of 8.07 liters. This cow, after five years of continuous lactation without freshing, produced milk containing considerably more ascorbic acid than the average for the other cows in the herd (18.17 mg./l.), although receiving the same rations, housing and management. F.J.D.

32. The Vitamin D Content of English Butter Fat Throughout the Year. K. M. HENRY AND S. K. KON, Natl. Inst. Res. in Dairying, Univ. Reading. Biochem. Jour., *36*, No. 5 and 6: 456. 1942.

The values for the non-saponifiable residue varied from less than 0.1 I.U./g. fat in the months November–March to 0.55 I.U./g. in July and 0.97 I.U./g. in August. On the whole, there was good correlation between the vitamin D content of butter and the amount of sunshine to which the cows were exposed. V.C.S.

33. The Influence of Lactose and Its Hydrolysis Products on the Absorption of Calcium. EUGENE ROBERTS AND A. A. CHRISTMAN, Dept. Biol. Chem., Med. School, Univ. Mich., Ann Arbor. Jour. Biol. Chem., *145*, No. 1: 267. 1942.

Under the conditions of these experiments, the presence of lactose or its hydrolysis products, glucose and galactose, did not increase the rate of calcium absorption. In higher concentration, lactose apparently inhibited calcium absorption. V.C.S.

34. Digestion Characteristics of Various Types of Milk Compared with Human Milk. F. J. DOAN AND J. L. DIZIKES, Pa. Agr. Expt. Sta., State Col., Pa. Milk Plant Monthly, *31*, 9: 24–28, 44.

In-vitro digestion studies were made on several types and varieties of milk in order to compare their digestibilities with that of human milk. For this purpose latex bags were used in which peristaltic movements were effected which simulated those of the infant stomach. After three hours of gastric digestion the curds were separated by different screens and analyzed for total nitrogen. The comparative digestibilities of the various milks were found to be in the following order: breast milk; lactic acid milk buttermilk pH 4.0 to 4.5; superheated soft curd milk; evaporated milk; superheated soft curd milk, homogenized; lactic acid milk pH 5.4 to 5.7; boiled milk; homogenized milk; sonized milk; metaphosphate treated milk 0.1%; chocolate milk; trypsin treated milk 0.008%; goat's milk; pasteurized milk; raw milk; and locust bean treated milk 0.1%. The lack of any close relationship between curd tension and digestibility was found. These results indicate that the curd tension value is of very doubtful significance in predicting the digestibility and curd character of milk. G.M.T.

35. Thiamin Content of Milk in Relation to Vitamin B_1 Requirement of Infants. ELIZABETH M. KNOTT, Dept. Ped. Univ. Chicago, Chicago, Ill. Amer. Jour. Pub. Health, *32*, 1013–1017. 1942.

The thiamin content of milk is important since it is the only natural source of vitamin B_1 available to the young infant. The average thiamin content of 18 samples of pasteurized milk was 26 micrograms per 100 ml. as compared to 24 μg. per 100 ml. in the case of 16 formulae prepared from pasteurized milk which had been boiled for 3 minutes. The range of thiamin for the pasteurized and boiled milks was from 18 to 35 μg. per 100 ml. Twenty-five commercial samples of evaporated milk representing 7 different brands had a slightly lower range from 13 to 27 μg. of thiamin with an average value of 19 when calculated on the basis of reconstitution with equal parts of water. Samples of breast milk from 17 different women ranged only from 3 to 18 μg. per 100 ml. of milk with an average of 9 μg. A footnote added to the paper at the time it went to press states that additional data shows breast milk thiamin values to be higher. Average thiamin content for women successfully nursing young infants was 20.1 μg. per 100 ml.

Destruction of vitamin B_1 due to the processing of evaporated milk ranged from 23 to 35% for the four lots of milk tested. The amount of further destruction during storage varied with different milks reaching 50% after one year for two milks. Another milk showed loss of one-half its original vitamin content in two months time.

An intake of about 80 units of thiamin daily meets the infant's immediate needs and above this, an excess begins to appear in the urine.

Since cocarboxylase is the functioning form of thiamin in the body cells, its estimation in blood cells is an approach to the problem of thiamin requirement. Based on several hundred determinations, about 5 μg. of cocarboxylase per 100 ml. of blood was found to be a normal value for health. A fairly satisfactory level of 3–4 μg. can be furnished by milk formulae but during times of stress supplementary thiamin is desirable as a safety factor.

M.W.Y.

ICE CREAM

36. Using Fruit Purees to Get New Flavors in Ribbon Ice Cream. DONALD K. TRESSLER. Food Inds., *14*, No. 9: 49–51, 99. Sept., 1942.

Pectinized fruit purees used in variegated ice creams make possible many unusual fruit flavors. Each of the fruit flavors or purees described in this paper has been successfully used in experimental batches of ice cream and several are already in commercial use.

Ribbon or revel ice cream has made possible many new types of ice cream and have made others more popular. Gives details on making these

ice creams and consistencies of both the puree and the ice cream. Enzyme converted corn syrup is best for making the puree as it produces better qualities.

Details for making the fruit flavors and using them in ice cream are given. J.C.M.

37. Use of Soya Lecithin Will Save Scarce Fats. JOSEPH STANLEY. Food Inds., *14,* No. 7: 69–71. July, 1942.

This ingredient will also extend the stability of fats. It will reduce the amount of egg yolks required in ice cream, will stabilize vitamin A potency and extend vitamin B_1 activity and improve quality in the confectionary, baking, and ice cream industries.

Lecithin serves as an interforce agent, a reducer of friction or attraction between particles, thus greatly changing the properties and qualities of foods. Purified hexane is used to extract the lecithin and oil from soybeans. During recovery the solvent is distilled off from the extract leaving lecithin and oil. The lecithin is removed by hydration with steam and is dried under vacuum at low temperatures. Characteristics of lecithin and their applications are listed.

In confectionary it is used as an emulsifying agent. In ice cream it has a special stabilizing effect; gives a velvety smoothness, improves body, melt down and heat shock characteristics. It also prevents crystallization.

J.C.M.

38. A Method for Saving Sugar in Ice Cream. ALAN LEIGHTON AND OWEN WILLIAMS, Div. Dairy Res. Labs., Bur. Dairy Indus., Washington, D. C. Ice Cream Field, *40,* No. 3: 14. 1942.

According to the authors there are only 5 components of ice cream other than flavor and stabilizer which are: butterfat, milk-solids-not-fat, sugar, water (including ice) and air.

Their results showed that mixes containing 13 to 16% sugar were satisfactory in sweetness and that this represented a sugar to water ratio of 1–5 to 1–4. Subsequent experiments were conducted using 13% sugar as the standard, that is the sugar to water ratio was maintained at 1–5, even though the milk solids and sugar contents of the mix were varied and the overrun was adjusted so as to maintain a uniform food solids per gallon in the finished ice cream. The results obtained show that sweetness of ice cream depends upon the concentration of sugar in the water phase of the mix, rather than upon the ratio of sugar to the total weight of the mix. The authors state, "... although each mouthful of ice cream contained the same weight of sugar and the same weight of food solids the sugar was used with greater sweetening effect as the water content of the mixes decreased." In

other words it is claimed that decreasing the water content of the mix is equivalent to increasing sweetness. When this practice is followed the authors recommend taking higher overruns to offset increased costs and "to obtain a better product."

It is stated that the present sugar shortage is not sufficient to make the substitution of milk solids for sugar an economical practice, but this practice may be followed if the situation becomes more acute. W.C.C.

39. Substitutes for Cocoanut Oil in Chocolate Ice Cream Coatings. J. J. SHEURING AND P. H. TRACY, Dept. Dairy Husb., Univ. Ill. Ice Cream Trade Jour., *38*, No. 10: 64. Oct., 1942.

"Ice cream chocolate coating contains 50 to 60% fat, 25 to 40% sugar and 0.2 to 0.3% lecithin. If 10 to 12% milk solids are added the product is termed a milk chocolate coating. Cocoanut oil is frequently added to increase the spreading ability of the coating and to give brittleness to the finished product.

"A number of experiments were conducted in an attempt to find domestic oils and fats that could be used as substitutes for cocoanut oil in chocolate coatings. An effort was also made to make a chocolate coating from cocoa, powdered sugar, and domestic oils and fats that would be satisfactory for emergency use. Soybean oil, hydrogenated soybean oil, coso oil, beef fat, hydrogenated cottonseed oil, and corn oil were used as substitutes for cocoanut oil. Using a standard milk chocolate coating as a control, samples were prepared by diluting the coating with 8 to 25% domestic oils and fats. The results indicate that 8 to 15% of low melting point fats and 10 to 25% of high melting point fats can be used successfully as substitutes for cocoanut oil. The best product was made by using a combination of 5% added hydrogenated soybean oil and 5% soybean oil.

"A coating satisfactory for emergency use was made using 10% cocoa, 40% powdered sugar, 35% hydrogenated soybean oil and 15% soybean oil. This coating is not as satisfactory as products prepared from chocolate liquor but may be used if more stringent conservation of butter, cocoanut oil, and chocolate is necessary." W.H.M.

40. Flavor Scoreboard. EDITORIAL. Ice Cream Trade Jour., *38*, No. 10: 16. Oct., 1942.

The results of a survey conducted by the Ice Cream Trade Jour. showed that ice cream manufacturers have greatly reduced the number of flavors of ice cream and novelties. The average number of flavors was reduced from 16.5 in July, 1941, to 7.5 in July, 1942, and the average number of novelties was reduced from 8.1 to 4.3. W.H.M.

41. Forty Percent Mileage Reduction. CLARENCE M. SWITZER. Ice Cream Trade Jour., *38*, No. 10: 34. Oct., 1942.

The General Ice Cream Co. operating some 1200 trucks in 80 locations engaged in delivery of ice cream, retail and wholesale milk has had a combined saving of 40% in mileage as compared with last year. This accomplishment was made by transfer of customers between plants, use of horses in some large downtown markets, greater use of common carriers and reduced deliveries to customers. W.H.M.

42. What Is the Wartime Outlook for Flavoring Materials? LLOYD E. SMITH, Pres., Flavoring Extract Mfrs. Assoc. U. S. Ice Cream Trade Jour., *38*, No. 10: 46. Oct., 1942.

It is estimated that Mexico will not be able to supply more than 50% of the 1,000,000 pounds of vanilla beans used annually in the United States. Since importations from Madagascar have been cut off or are uncertain, the deficiency will have to be made up by the use of compound and imitation vanillas made from vanillin and coumarin which are also scarce.

Essential oils although made mostly from imported raw materials which have been cut off are now being made from herbs grown in this country and supplemented by imitation oils.

Citrus juices have been frozen by the government, however, there is enough orange and lemon oil available for our flavor needs for sometime. Rationing of cocoa beans has caused a marked reduction in the amount of chocolate flavoring available. At the present time the amount available is 60% of last year. W.H.M.

43. How the Industry Is Slashing Mileage. EDITORIAL. Ice Cream Trade Jour., *38*, No. 10: 14. Oct., 1942.

A nation-wide survey made by the Ice Cream Trade Jour. of delivery operations of ice cream companies with a 1941 gallonage of 51,932,983 revealed a 32.5% reduction in mileage in July, 1942 over July, 1941. By applying this percentage to the entire industry, the mileage reduction for the month was over 4,400,000 miles.

On the basis of the saving in gasoline consumption, the reporting companies showed a 26% saving in mileage for the first seven months of 1942. In making this saving 39.3% of the reporting companies indicated that it was necessary to eliminate some dealers and 97.1% found it necessary to install additional cabinet equipment. A few companies were found to drop delivery personnel, however, 84.4% of the companies were able to use former delivery employees in other departments. W.H.M.

44. Stretching Your Supply of Coating. J. H. ERB, Dept. Dairy Technol., Ohio State Univ., Columbus. Ice Cream Trade Jour., *38,* No. 10: 26. Oct., 1942.

It is important when purchasing chocolate coating to know its composition, especially the chocolate solids-not-fat and total fat. The fat content is usually 55 to 65% and the minimum solids-not-fat content necessary for the proper flavor and color is about 5 to 6%. Some coatings contain up to 15% chocolate solid-not-fat. Coatings containing cocoa fat have a melting point around 92° F. Other fats may be substituted. Vegetable fats, unless they are hydrogenated to some degree, have melting points too low to make them entirely satisfactory. Corn oil, due to its low melting point, when subjected to warm temperature develops an undesirable greasiness, dries slowly and is subject to bleeding. The melting point of the oils should not be too high. By mixing high and low melting point fats a desirable melting point of 80° to 85° F. may be obtained.

The viscosity of the coating should be standardized to insure uniform coverage.

Lecithin, a derivation of soybeans added in small amounts to coatings compounded from cocoa, sugar, and oil is very helpful in producing a homogenous mass and in retarding moisture thickening.

Ice cream manufacturers who are unable today to buy a sufficient quantity of ready-made coating, may be able to purchase confectionery type coating and modify it. Such coating will contain about 42% chocolate liquor, 43% sugar and 15% cocoa butter. Since the chocolate liquor contains about 50% fat, the coating will contain about 35% fat and 21% solids not fat. For ice cream coating the manufacturer can add to every 60 pounds of confectioner's coating 40 pounds of fat or oil, thus stretching a 60% quota to 100% of previous requirements. Further stretching can be obtained by adding more fat, powdered sugar, milk solids and lecithin. This can be done by melting the slab coating and added fat, placing them in a batch ice cream freezer and sifting the dry ingredients in as the dasher revolves, and mix for about 10 minutes. Coatings consisting of fat, sugar and milk powder now on the market may be mixed with confectioner's coating, or a fair coating can be made from cocoa, sugar, hydrogenated oil, milk solids and lecithin. W.H.M.

45. Data and Regulations in Use of Egg Yolk. B. I. MASUROVSKY. Food Indus., *14,* No. 4: 65–98. April, 1942.

Egg yolks are rich in nutritive elements, act as an emulsifying agent, give distinct flavor and save on sugar. Regulations covering use in ice cream are cited and practical data given on quantities needed are included. Egg yolk aids in the whipping of ice cream which gives a smooth texture. French type ice cream uses more yolks and this custard blends with milk and cream

of the frozen product. Used in other frozen desserts they save sugar as they would not be as sweet without this rich and flavorsome ingredient. Regulations for using eggs in ice cream are listed. J.C.M.

46. How to Calculate Gallon Weights of Dairy Products. A. J. HAHN AND P. H. TRACY. Food Indus., *14,* No. 6: 55. June, 1942.

Simple and accurate method of computing weight per gallon of ice cream mixes, condensed milk and other products are given. Procedure and table of weights per gallon as measured and calculated constitute a valuable addition to this article. J.C.M.

MILK

47. Some Cooking Qualities of Homogenized Milk. I. Baked and Soft Custard. R. E. CARR AND G. M. TROUT, Mich. Agr. Expt. Sta., East Lansing. Food Res., *7,* No. 5: 360. Sept.–Oct., 1942.

The cooking time for custards made with homogenized milk was found to be from 15 to 20 minutes longer than when made with unhomogenized milk due to slower heat penetration. Baked custards exhibited stiffer gel formation and less syneresis when made with homogenized as compared with unhomogenized milk, immediately after removal from the cup and after three hours standing. Soft custards containing homogenized milk were more viscous than those containing unhomogenized milk.

Judges showed no preference in palatability between the baked custards but a marked preference was exhibited toward the soft custards made with homogenized milk as compared with those made from unhomogenized milk.

The authors note that their findings do not substantiate those of Hollender and Weckel (Food Res., *6,* No. 4: 335, July–Aug., 1941.), but point out that the conditions of the experiments differed considerably. F.J.D.

48. How to Prevent and Remove Milk Deposits (Part II). LEWIS SHERE. Food Indus., *14,* No. 7: 63–66. July, 1942.

Constant attention to proper cleaning routine, involving the use of suitable milkstone removing products and effective cleaning solutions combined with the use of softening agents for hard water, if present, will remove milkstone deposits and prevent their subsequent deposition.

Rinsing equipment right after use will reduce deposits. Cold water is best. Everyday cleaning with the aid of proper water softening properties to take care of hard water salts help. Smooth equipment prevents milk from adhering.

Conditions of milk deposits and other contamination are of two kinds: (1) That built up over a period of time, (2) Milkstone built up every day. Removal of type 1 is usually done by a product acid in nature, gives agents

and methods used. Removal of second type can usually be done by special removers, gives details on removal and procedure.

Milkstone removers for milk cans are explained and discussed. Also the coin test which is simply scraping the equipment with a coin to see if there is any deposit left, is mentioned. Instructions on removing materials are given. J.C.M.

49. Seasonal Variation in Production in the New York Milkshed, and Its Relation to Production-Adjustment Plans. A. J. POLLARD. N. Y. Agr. Expt. Sta. Bul. 783. June, 1942.

The purpose of this study was twofold, (1) to measure differences in seasonal and total milk deliveries by various groups of producers in the New York milkshed, and in the butterfat content of this milk, and (2) to determine how price returns to the different groups would be affected by certain modifications in method of paying producers, designed to adjust milk supply either seasonally or annually. The study was based on records of more than 3000 representative dairies in the area over a 3-year period, (July, '36, through June, '39).

Considerations of space do not permit the inclusion here of the rather voluminous summary and conclusions. The most outstanding facts noted were (1) that producers whose output was more or less uniform throughout the year (those with even or winter dairies) received higher price returns than those who produced the bulk of their milk in spring and summer (uneven or summer dairies), and (2) the greatest price differential in favor of the uniform producers resulted from a base-rating plan which used the months of Sept., Oct., and Nov. as the quota-forming period. J.G.A.

50. How One Dairy Is Conserving Its Equipment. OLIVER N. GEHL, Milwaukee, Wis. Ice Cream Trade Jour., *38*, No. 10: 28. Oct., 1942.

Since adopting alternate day delivery of milk, the author states that this system has resulted in a 30% reduction in daily mileage and gasoline consumption. This saving in man power and delivery cost has been nullified by increased cost of milk, higher wages and taxes.

Cleaning operations in the plant have been changed from a night job to one which is done immediately after the last day time operation. To prevent waste in the use of detergents the amount used is reduced to a minimum for thorough cleaning. A greasing and maintenance record is kept on all motors and reserve motors are mounted on bases so that replacements can be made without delay. A check on spare parts is made and an inventory taken. Daily mileage, gas, oil, and repair records are kept on all trucks. Oil is drained every 1000 to 1500 miles and reclaimed. W.H.M.

51. Acid Detergent Makes Cans More Sanitary. F. M. SCALES. Food Indus., *14*, No. 4: 51–53. April, 1942.

Cans washed cleaner at lower cost with acid detergent. Lathrop Paulson developed a new can washer and installed it at a Sheffield Farms Company milk receiving station. A month later the can inspector said it gave the cleanest cans he had ever seen and also that they had no odor even after standing. Best results obtained by using special acid and washer. Data on tests made for the solutions and methods used and results are listed.

An advantage of this system is the wetting agent used. It promotes drainage and dries freely leaving a perfectly dry surface. Acid cleaned cans are odorless while alkali are rarely so.

The acid cleaner will be best for both aluminum and laquered cans which will be used after the war. The same acid cleaner can be used for sterilizing bottles, dish washing and plant equipment with reduced costs. J.C.M.

52. The Future of Creameries. CLIFFORD SKERTCHLY. Food Mfr., *17*, No. 9: 247–249. Sept., 1942.

England's creameries do not have the use of milk that they used to and are not prospering as well as they were before the war. The demand for liquid milk is greater than the supply and many of the creameries have shut down. The position of the creamery industry is expected to become still more serious during the remainder of the war. Milk production is a major operation but only for liquid milk. After the war they will be in a similar situation and the government can offer no help. The creamery industry appears doomed in England. J.C.M.

53. How to Prevent and Remove Milk Deposits. I. LEWIS SHERE. Food Indus., *14*, No. 6: 44–46. June, 1942.

The nature of milk deposits on equipment and utensils used in processing and handling milk products depends upon the product being processed and the process being employed. Upon the nature of the deposits depends the methods used to prevent deposit formation or if formed to effect removal.

Milkstone is any contamination on dairy equipment that defies routine daily cleanup operations. Milk film can be removed every day. Composition of milkstone and data on milk deposit. Objections to milkstone: (1) Source of high bacteria count, (2) good insulator, (3) may cause off flavors, (4) unsightly.

Composition of milkstone is effected by the following factors: (1) Rate of flow through equipment, (2) rate and extent of heating, (3) rate of cooling and temperature from which milk is cooled, (4) amount of products handled, (5) kind of product processed, (6) type of equipment.

Additional factors not directly connected with products are: (1) Effi-

ciency of cleanup, (2) hardness of water, (3) water softening action of cleaner and sterilizer, (4) condition of surface of equipment, (5) routine in rinsing and sterilizing. J.C.M.

54. Depreciation Rates on Milk Processing Equipment. ANONYMOUS. Milk Dealer, *31,* No. 11: 46–47. Aug., 1942.

The latest depreciation and obsolescence estimates on milk-processing equipment of the Bureau of Internal Revenue, revised to 1942. Estimated average useful life of milk-processing equipment, in years, has been in practically every instance lengthened by the revenue department over the 1931 estimates of the Depreciation Committee, International Association of Milk Dealers.

Soaker type bottle washers in 1931, for example, were assigned an estimated life length of 10 years by the Depreciation Committee. In 1942, however, the revenue bureau estimates length of bottle washer life as 16 years. Bottle filler and capper life length estimate was raised to 12 years from the 1931 estimate of five years, and homogenizer life was increased from 12½ years to 17 years.

Estimated life of equipment in fluid milk and ice cream plants, the revenue bureau reports in Bulletin "F," is 15 years. For outside equipment, the following lives are recommended: Trucks, 4 to 8 years; truck tank cars, 10 years; horses and harnesses, 7 years; milk bottle cases, 4 years; milk cabinets, 6 years; milk cans, can jackets, 4 years; railroad tank cars, 25 years.

Revenue bureau's table of average useful life in years on ice cream and bottled milk plant equipment is given. C.J.B.

Editor's Note: For complete information refer to Bulletin "F," Income Tax Depreciation and Obsolescence Estimated Useful Lives and Depreciation Rates. U. S. Treasury Dept., Bureau of Internal Revenue. Price, 15 cents.

55. This Problem of Delivery and Sales. ANONYMOUS. Milk Dealer, *31,* No. 12: 28–29. Sept., 1942.

A discussion of how the Poinsettia Dairy Products Corp. of Tampa, Fla., met the delivery problem under war conditions. This dairy has adopted every-other-day service. It maintains early morning delivery for the following reasons:

1. The milk is delivered in time for breakfast when the majority of the day's supply is consumed, reducing the need for storage facilities to the minimum.

2. The delivery is made at a time when all customers are at home and will be taken in before the temperature of milk rises very high.

3. The double load of milk can be delivered with only a 20% increase in delivery time.

4. Collections on bicycles can be made in most cases in less time than it took to collect with trucks.

5. It is possible to give men one full day off each week, and still have more time for solicitation of new customers and yet maintain the same number of routes and men as formerly had.

The results obtained are:

Every-other-day deliver and collecting on bicycles has reduced truck mileage 48%.

Retail collections for the first quarter of this year have averaged 99.2%.

Retail sales for May of this year were 6.5% better than our peak winter month and 28.5% better than the same month last year. C.J.B.

56. A Simple Milk-Elevator. W. G. WHITTLESTON, Anim. Res. Sta., Wallaceville, Australia. New Zeal. Jour. Sci. and Technol., *23,* No. 1B: 17B–23B. 1941.

A vacuum-operated elevator for lifting milk, without turbulence, from the milk-pipe of milking machines is described. The device maintains a steady vacuum in the milk-pipe regardless of rate of milk flow. W.C.F.

MISCELLANEOUS

57. Food Nomenclature in Wartime. THOMAS MCLACHLAN. Food Mfr., *17,* No. 8: 225–227. Aug., 1942.

Food substitutes controlled by the government in England must have certain qualities in specific articles. He lists substitutes or foods whose contents have been changed since the war. Problem is to know whether to change the name of the products which are inferior to those of pre-war days. Many foods have become degraded to such an extent that one wonders what new changes will have to be made. Lists of such foods are given. Wartime flavors are called essence and not substitute. Author thinks problems should be settled soon for the well being of the general public. J.C.M.

58. Food Packing Research. D. W. GROVER. Food Mfr., *17,* No. 7: 185–187. July, 1942.

Tests have been made on modern packaging and requirements for these are listed. The author gives details on moisture permeability and the testing materials go through to determine it.

Impurities which may occur in wrapping are: (1) bacteria, mold, etc., (2) chemical ingredients of wrapping materials, (3) chemical impurities of wrapping materials. Proper treatment of paper pulp may prevent molding. Sanitary conditions should be employed where paper and board are made for food trade.

Wrapping materials should be sterilized. Paraffining is good. Names and details about impurities are given. Antiseptics have been used for treating wrappers, and transparent film for fatty foods is undesirable since light will cause rancidity. Colored wrappers should be tested before use in this field. Foils are good but metal is scarce. J.C.M.

59. Stoneware Pumps for Food Industry. JOHN DESMOND. Food Mfr., *17*, No. 7: 193–194. July, 1942.

Lists important factors for precise conditions of service in pumps. There is need for absolute purity of the product used in the pumps where the liquid comes in contact with it. It should be resistant to weak acids and other chemical constituents.

Copper and iron produce tallowy flavors in milk products. Iron causes discoloration in fruit juices, and aluminum has an effect on flavor but it is not known if it has any toxic effects.

Chemical stoneware is being used more and more because it saves metals and has absolute resistance to all acids and other corrosive chemicals. As far as food products are concerned it is completely inert. In the last few years great improvements have been made. One grade has a very good resistance to thermal shock. Some pumps are now in use with a stoneware interior and metal exterior. J.C.M.

60. Wartime Packaging. Food Mfr., *17*, No. 5: 133–134. May, 1942.

Government regulations rule packaging today. Their appearance will be simplified and only necessary products packaged. Discusses shortage of paper, tin and glass in England and what has been done about it.

Blackplate metals are not suitable as yet since a good laquer has not been developed and the metal is hard to solder. Brunofix process is discussed. J.C.M.

61. Refrigeration Engineering in Emergency Cold Storage. Food Mfr., *17*, No. 5: 129–132. May, 1942.

Government cold stores have been erected to preserve food in case of abnormal conditions and to meet war needs. Sites for these food centers have been carefully selected. Gives plans for construction of these buildings. Each unit is self contained. Each has own electrical supply and engine room. Gives details on compressors and explains the whole refrigerating system. J.C.M.

62. The Contamination of Foods by Poison Gases (Part I). W. R. WOOLDRIDGE. Food Mfr., *17*, No. 4: 96–100. April, 1942.

Dangerous contamination can be achieved by high concentration of gas liberated into the immediate vicinity of an exploded gas bomb or from gas

spray from a low flying plane. The effects of contamination are nausea and chronic ailments of the alimentary system, sometimes resulting in death.

Extent of contamination depends upon food composition and nature of gas. Watery foods absorb more gas. Fatty or dry foods absorb mustard gas readily. Foods should be wrapped and kept in metal containers.

Decontamination is achieved by: (1) Aeration, (2) washing, (3) removal of contaminated surface layer, (4) suitable cooking, (5) dilution by admixture with similar but unaffected foodstuff, (6) appropriate chemical treatment, (7) weathering, (8) application of 2 or more above methods. Each method is explained in detail.

Foods contaminated can usually be found by their smell. Some cannot, however, and should be analyzed if suspected.

Lung irritant gases can usually be removed by aeration for 24–48 hours. Lists are given of the ways of removing gases from food.

Discussed foodstuffs affected by tear gases, nose irritant gas, mustard gas, and Lewisite and their decontamination. J.C.M.

63. The Contamination of Foods by Poison Gases (Part II). W. R. WOOLDRIDGE. Food Mfr., *17*, No. 5: 123–126. May, 1942.

Continues with explanation of detection and identification of war gases and how to analyze for them. Goes into detail and lists many references. J.C.M.

64. Water Treatment. R. B. MCNEAR, Internatl. Filter Co., Chicago. Natl. Butter and Cheese Jour., *33*, No. 10: 18. Oct., 1942.

The water problem should be diagnosed by a competent water chemist. The cost and methods of improving the supply will depend on the physical and chemical characteristics of the water: dirty water is filtered by pressure or gravity filters; turbidity is removed by coagulant; palatability is improved by activated carbon; sterilizing is accomplished by chlorination of cleaned water; dissolved iron which may enter the water supply in various ways requires special treatments; hard water may be softened by zeolite or lime-soda treatments. Other special treatments may be necessary for water containing dissolved minerals or organic matter, especially when it is to be used for a process like butter washing. Costs of treating water will vary with water characteristics and amount treated. A hypothetical amount of 3,720,000 gallons used per year is taken as the basis of a table showing estimated costs for each of the above treatments. Total of operating and fixed charges per million gallons are: for chlorination, $15.17; filtration, $20.54; taste and odor removal, $29.10; iron removal, $42.78; zeolite softening, $93.70. Costs of combinations of these treatment effects are listed, the highest cost being for demineralization (equivalent of distilled water), $500 per million gallons. W.V.P.

65. Outlook Serious for Metal Containers—Will Improve in 1943. L. V. BURTON. Food Indus., *14*, No. 4: 41–43. April, 1942.

A moderate amount of steel containers is available. A change from tinless steel to bare or enameled may occur.

Delays are caused by installation of new type machinery and testing of new materials. Bonderized plate is a new metal used for cans and only one plant at Gary, Ind., is producing it. Others may have the new plate by autumn. It is made by coating steel sheets with iron phosphate.

The tin supply is believed ample to carry food industries until new plants can be developed. The tin can salvage is helping, and some is still coming from South America. Amount of tin per can will be reduced.

The Bonderizing process uses zinc dihydrogen phosphate and there is enough of that to cover the need. Methods for cleaning before soldering must be found. New metals must be safe. Functions of Bonderized film are: (1) to secure adequate adhesion of organic films, (2) to eliminate underfilm, (3) to reduce corrosion in the organic film.

Oils for enameling are hard to get. Bonderized plate must be given at least one coat of an organic enamel.

Fruit canners must have some tin on their plate and this can be deposited in minute quantities by an electrolytic process. Research is under way to find if Bonderized will stand acids.

Steel supplies are definitely limited and experiments are being carried on to find how to cut down the amount of steel in a can. To sum it up, the metal container problem is not as acute as was believed but it will be a period of months before substitute containers can be produced. J.C.M.

66. Wrappers Tested for Moisture Loss. GORDON W. MCBRIDE. Food Indus., *14*, No. 4: 46–47. April, 1942.

Use of cellophane and metal foils has been greatly restricted. The National Bureau of Standards tested packages used for cigarettes and these results can be used for food packages. Many varieties of packages were used. Unsealed packages lost moisture very rapidly. Tables for moisture loss and procedure for testing for moisture loss are given. J.C.M.

67. Foods Need Guarding From Gas and Glass. ERIC HARDY. Food Indus., *14*, No. 5: 37–38. May, 1942.

Air raids may ruin food by filling them with flying glass particles; or if gas is used food may be contaminated by it and rendered unfit for consumption. Precautions taken in England to protect food and methods used for decontaminating gased foods are discussed.

Food should be kept in air-tight containers and under cover. Store window displays should be reduced. The glass is dispersed in very freakish manners by a bomb or mine and can even penetrate walls. Food in tin cans

alone is not safe. Food should be dispersed over an area so that not all would be destroyed.

Fats absorb gas. Liquid gas should be swabbed away if possible. Fats should be placed in large metal containers and sealed with adhesive tape. A teacup or tumbler should be inverted over the milk bottle. Cereals, eggs and sugars should be put in metal containers. Meat after contamination can be aired but some will be destroyed.

Water may become contaminated and chlorine is the easiest way of purifying it. J.C.M.

68. **Food Official Reviews Wartime Problem of Bottlers.** HERMANN C. LYTHGOE, Food Indus., *14*, No. 6: 47–48. June, 1942.

Because of the shortage of sugar and tin the opinions of a food official relative to the objectionable character of saccharin and of copper contamination are important. The author suggests ways to get adequate sweetness even under sugar rationing.

Saccharin has no food value and may be injurious to health. He discusses saccharin in beverages and as a food. Author advocates low sugar production and advocates substitution of corn sugar and less citric acid. The copper content of carbonated beverages is discussed and also inspection of bottling plants. Bacterial content studies also are recorded. J.C.M.

69. **Teaching Farm Sanitation as an Easier Practice.** DAVE NUSBAUM, Dept. Dairy Indus., Univ. Wis., Madison. Milk Dealer, *31*, No. 12: 31–32. Sept., 1942.

The author points out that farmers as a group can never be satisfactorily regulated into producing milk properly. They must be shown more reason for doing certain things than the mere fact that it is a requirement of the city board of health. The importance of considering human nature and of being practical-minded in teaching farm sanitation is stressed. C.J.B.

70. **The Cleaning of Dairy Equipment. Part I. Cleansers for Use in Dairy Factories.** F. H. MCDOWALL, Dairy Res. Inst., N. Z. New Zeal. Jour. Sci. and Technol., *23*, No. 3A: 146A–154A. 1941.

A general discussion of detergents is given, with formulas for cleansers. Sodium carbonate is especially recommended as an ingredient. W.C.F.

71. **The Cleaning of Dairy Equipment. Part II. Cleaning Operations in a Dairy Factory.** F. H. MCDOWALL, Dairy Res. Inst., N. Z. New Zeal. Jour. Sci. and Technol., *23*, No. 3A: 154A–165A. 1941.

Cleaning operations in dairy plants are discussed. W.C.F.

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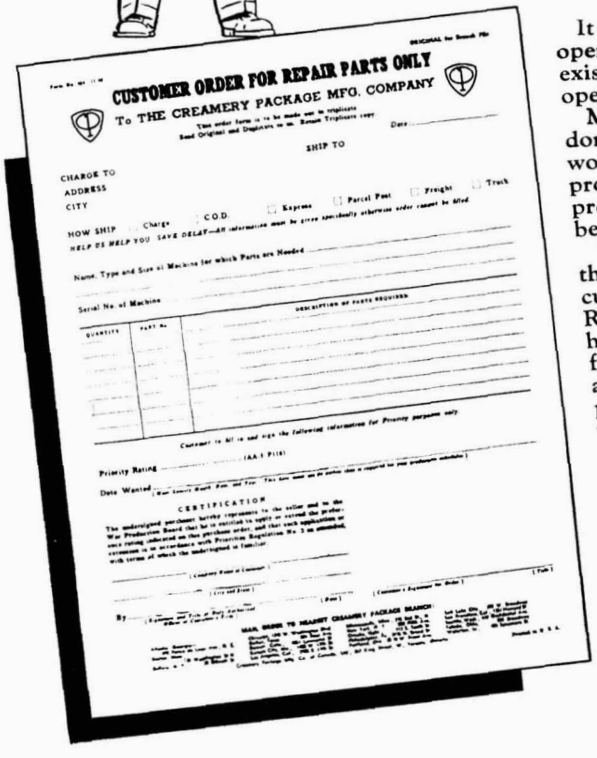

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