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## THE KEEPING QUALITY, SOLUBILITY AND DENSITY OF POWDERED WHOLE MILK IN RELATION TO SOME VARIATIONS IN THE MANUFACTURING PROCESS. I. KEEPING QUALITY[1]

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The volume of whole milk powder manufactured in the United States greatly increased during World War II. This product has not always been well accepted because under certain conditions it is subject to chemical or physical deterioration which may affect its palatability and reconstitutability in a relatively short period of time.

Many of the basic factors involved in the appearance of storage defects in whole milk powder are well known. However, further study is necessary to increase existing knowledge of the causative factors and thus to increase the shelf-life and consumer acceptance of whole milk powder.

### REVIEW OF LITERATURE

A review of the literature indicates that high preheating temperatures of milk improve the keeping quality of powdered whole milk. The treatments reported as giving the best results varied considerably in the temperature-time ratio employed. The following heat treatments have been reported as beneficial in the production of whole milk powder of good keeping quality: 170 to 181° F. for 30 minutes (10, 11, 13, 24); 175° F. for 15 minutes (15); 190 to 195° F., without statement regarding time of exposure (20); 190° F. for 20 seconds, followed by a holding period of 2 to 3 minutes at a slightly lower temperature (17, 22); 220° F. for 10 seconds (15); and 250° F. for 1 second (24).

The reasons given for the effectiveness of the high preheat treatment by the above workers included: (a) the production of reducing compounds, namely sulphydryls, and (b) more complete inactivation of enzymes. The

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production of sulphydryls through high preheat treatment is indicated by the work of other authors (6, 7, 16).

It has been shown that lecithin is easily oxidized, yielding a tallowy flavor (16, 23). In 1902 it was reported that temperatures of 203 to 230° F. destroyed as much as 26 per cent of the lecithin in milk (1), which may partially explain the improved keeping quality of whole milk powder made from high preheat-treated milk. Separate preheat treatment of cream and skim milk is associated with the fat and more particularly the lipids (20). Further evidence that lecithin may, in part, be responsible for the development of oxidized flavor has been produced by removing approximately one-half of the lecithin prior to powdering, which resulted in a better keeping powder even though the preheat treatment was only 160° F. for 30 minutes (2, 3).

Vacuum condensing of the milk improved the keeping quality of the powdered whole milk, apparently by removal of volatile catalysts (12). Condensing the milk to a high concentration was reported as desirable in the production of better-keeping powdered whole milk (24), and an increasing concentration of the milk—31, 38 and 45 per cent total solids—was shown to result in less retention of oxygen by the powder and an improved keeping quality (9).

All of the literature is in agreement that a low storage temperature improves the keeping quality of whole milk powder (4, 5, 8, 10, 14, 15, 17, 18, 19, 21). For instance, it was shown (4, 5) that, although little difference in the keeping quality of the whole milk powder resulted when the storage temperature range was 39.2 to 53.6° F., a very marked impairment of the keeping quality was evidenced in powders stored at 98.6° F. A study (14) with whole milk powders containing 1.54 per cent moisture showed an improvement in keeping quality as the storage temperature was progressively decreased from 86 to 38.4° F. A straight line relationship was indicated in the range of 77 to 50° F. Below 50° F. the improvement in keeping quality was more marked for each 9° F. decrease in temperature, and at 38.4° F. the rate of oxidative deterioration was one-half that found at 77° F. Lea *et al.* (17) considered storage for one day at 59° F. equivalent to 6 hours at 98.6° F. or 3 hours at 116.6° F.

## EXPERIMENTAL PROCEDURE

The variables in the manufacturing procedure used in this study were limited to preheat treatment, precondensing and storage temperature. The four preheat-treatment levels studied were: 160 and 170° F. for 30 minutes and 170 and 180° F. for 10 minutes. For each run, morning milk in about 158-lb. quantities was obtained from the college herd and as nearly as possible from the same cows each day. Milk preheated at one of the levels indicated above was mixed thoroughly and divided into two equal parts.

One-half was then precondensed to approximately 20 per cent and the other to approximately 40 per cent total solids. The concentrated milk was homogenized at the condensing temperature and at 2,000 lb. pressure by means of a C. P. Multiflo homogenizer of 125-gallon capacity. The milk powder obtained from each lot of the concentrated milk was divided further into two lots and stored at 45 and 100° F.

The concentrated milk was atomized by air under 60 lb. of pressure and dried in an experimental spray drier. The spray nozzle was 1 mm. in diameter and was centered in the air outlet of 2 mm. diameter. The drying air, at about 255° F., flowed concurrently with that of the spray. The moisture-laden air was drawn from the drying chamber at approximately 160° F. through cloth dust collectors and then through a spray of cold water for dehydration by cooling.

The moisture content of the powder was determined by the vacuum oven method. Extremes in moisture content were 1.6 to 3.1 per cent, with most of the samples containing between 1.8 and 2.6 per cent moisture.

Immediately after drying the powder was manually mixed by means of a large spoon; 120-g. quantities were air-packed into no. 2 flat tins, hermetically sealed and stored at 45 and at 100° F. The powder was reconstituted on the basis of 1 part of powder to 7 parts of water and was scored by a panel of four judges 1 day after manufacture and at intervals of 1, 2, 4, 5, 6 and 10 months thereafter. For flavor scoring a previously unopened can was used. The following arbitrary scale of numerical values was used to rate the flavors: 1–2, bad; 3–4, poor; 5–6, fair; 7–8, good; 9–10, excellent. The "excellent" rating was awarded to samples free of any off flavors except for slight heated flavor. Whenever a heated flavor was detectable, the reconstituted milk was found not to be oxidized. The "good" rating was given to milk which was still quite acceptable in flavor, although it might have a very slight taint which could not be readily defined. The remainder of ratings were based on degrees of oxidized flavor.

## RESULTS AND DISCUSSION

The changes in average flavor scores of the milk powder samples are shown in table 1. The effects of preheat treatments on the retention of palatability in air-packed milk powder as brought out here are in general agreement with the effects observed by other investigators. The powders made from milk preheated at 170° F. for 10 and 30 minutes and at 180° F. for 10 minutes deteriorated little in flavor during a 10-month period of storage at 45° F., but powders made from milk preheated at 160° F. for 30 minutes deteriorated in flavor rather rapidly during storage at 45° F. Preheat treatment of the milk at 170° F. for 10 minutes is not as effective as the more drastic preheat treatments in inducing good keeping quality in the powder, and treatment at 160° F. for 30 minutes is very ineffective. When

stored at 100° F., all of the powders deteriorated rapidly in flavor and became oxidized in flavor in 1 to 2 months.

Hetrick and Tracy (9) found that increasing preconcentration of the milk (31, 38 and 45 per cent total solids) resulted in powders of better

TABLE 1

*Average flavor changes during storage*

| Powder from 20% concentrate | | | Powder from 40% concentrate | | |
|---|---|---|---|---|---|
| Age | Av. flavor score after storage at | | Age | Av. flavor score after storage at | |
| | 45° F. | 100° F. | | 45° F. | 100° F. |
| Preheat treatment—160° F. for 30 min.[a] | | | | | |
| 1 day | 7.7 | 7.7 | 1 day | 8.3 | 8.3 |
| 1 mo. | 7.2 | 5.7 | 1 mo. | 7.1 | 5.9 |
| 2 mo. | 8.0 | 4.3 | 2 mo. | 7.7 | 5.0 |
| 4 mo. | 7.0 | 2.8 | 4 mo. | 7.0 | 3.2 |
| 5 mo. | 6.4 | 2.5 | 5 mo. | 6.1 | 2.4 |
| 6 mo. | 7.0 | 3.1 | 6 mo. | 6.3 | 2.3 |
| 10 mo. | 5.5 | ..... | 10 mo. | 5.4 | ..... |
| Preheat treatment—170° F. for 10 min.[b] | | | | | |
| 1 day | 8.0 | 8.0 | 1 day | 7.9 | 7.9 |
| 1 mo. | 8.1 | 6.2 | 1 mo. | 8.4 | 7.0 |
| 2 mo. | 8.3 | 4.9 | 2 mo. | 7.9 | 5.4 |
| 4 mo. | 8.0 | 3.3 | 4 mo. | 7.7 | 3.9 |
| 5 mo. | 7.9 | 3.6 | 5 mo. | 7.8 | 4.6 |
| 6 mo. | 7.7 | 2.5 | 6 mo. | 7.5 | 3.6 |
| 10 mo. | 7.2 | ..... | 10 mo. | 7.3 | ..... |
| Preheat treatment—170° F. for 30 min.[a] | | | | | |
| 1 day | 7.8 | 7.8 | 1 day | 8.1 | 8.1 |
| 1 mo. | 8.1 | 6.2 | 1 mo. | 8.1 | 7.1 |
| 2 mo. | 8.2 | 6.1 | 2 mo. | 8.3 | 6.5 |
| 4 mo. | 7.6 | 3.1 | 4 mo. | 7.8 | 4.6 |
| 5 mo. | 7.6 | 3.3 | 5 mo. | 7.8 | 6.5 |
| 6 mo. | 8.0 | 3.3 | 6 mo. | 8.1 | 5.0 |
| 10 mo. | 7.8 | ..... | 10 mo. | 8.2 | ..... |
| Preheat treatment—180° F. for 10 min.[a] | | | | | |
| 1 day | 8.3 | 8.3 | 1 day | 8.6 | 8.6 |
| 1 mo. | 8.3 | 6.6 | 1 mo. | 8.3 | 7.3 |
| 2 mo. | 8.1 | 5.9 | 2 mo. | 8.3 | 6.9 |
| 4 mo. | 7.8 | 3.6 | 4 mo. | 7.8 | 4.9 |
| 5 mo. | 7.9 | 3.7 | 5 mo. | 8.1 | 4.7 |
| 6 mo. | 8.0 | 3.2 | 6 mo. | 8.1 | 4.4 |
| 10 mo. | 7.8 | ..... | 10 mo. | 8.2 | ..... |

[a] Each score is the average of 6 samples.
[b] Each score is the average of 4 samples.

keeping quality. From the results reported in the present paper, it appears that precondensing to 40 per cent total solids and preheat treating at 170° F. for 30 minutes or 180° F. for 10 minutes yields a powder with slightly better retention of palatability than does powder made from milk precon-

densed to the 20 per cent total solids level. The effect of concentration on the keeping quality was more marked when the powder was stored at 100° F. (table 1). However, at this temperature the improved keeping quality does not appear to be commercially significant.

## CONCLUSIONS

1. Preheat treatment of the milk at 170° F. for 30 minutes or 180° F. for 10 minutes resulted in powders of good palatability even after storage, air-packed, for 10 months at 45° F. Preheat treatment at 170° for 10 minutes was slightly less effective for producing powders of good keeping quality. Air-packed powders made from milk preheated at 160° F. for 30 minutes deteriorated rapidly when stored at 45° F.

2. Milks preheated at 170° F. for 30 minutes or at 180° F. for 10 minutes and precondensed to approximately 40 per cent total solids resulted in powders which retained slightly better palatability during 10 months of storage at 45° F. than did powder made from the milk precondensed to approximately 20 per cent total solids.

3. All air-packed powders stored at 100° F. rapidly became oxidized in flavor. The powders made from milk preheat treated at 170° F. for either 10 or 30 minutes and at 180° F. for 10 minutes and precondensed to approximately 40 per cent total solids deteriorated in palatability slightly less rapidly than did the powders made from the 20 per cent preconcentrate.

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# BENEFICIAL EFFECT AND ECONOMIC IMPORTANCE OF USING ALL COLOSTRUM PRODUCED IN CALF RAISING

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The high value of colostrum in the nutrition of the newborn calf has been well established (2), yet on most farms the colostrum not nursed by the newborn calf is wasted or not used for calf feeding. Practical methods of colostrum utilization must be devised and demonstrated to encourage its general use in raising herd replacements. This paper reports such a practical method.

Dann (4), in 1933, reported the vitamin A content of colostrum to be many times that of normal milk. Since then research workers have demonstrated repeatedly the importance of vitamin A in the nutrition of the newborn calf (5, 6, 8, 9, 10, 11, 13, 18). Colostrum also has been reported to be high in riboflavin (12, 15, 16), and a recent paper by Wiese *et al.* (17) indicates the newborn calf must have a dietary source of this vitamin.

In a recent paper on the physiological effects of extending the colostrum feeding period to seven days, the blood plasma level of vitamin A was reported to increase rapidly following the ingestion of colostrum, reaching a peak on the seventh day (14). The calves fed extra colostrum made more rapid weight gains and showed no signs of digestive disturbances during the colostrum feeding periods. The economic advantages of utilizing all colostrum in calf raising also were pointed out.

During the previous experiment, surplus colostrum was frozen and stored until used. On the average dairy farm this method for utilizing colostrum is not practical, since few farms are equipped to refrigerate and store any quantity of colostrum. Considerable inconvenience also is encountered in the feeding, since extra time and effort are required to prepare the colostrum for feeding.

The investigation presented in this report was undertaken to determine the effects of intermittent colostrum feeding for the duration of the milk feeding period. Observations were made of the effects on blood plasma vitamin A and carotene, weight gains and physical performance as indicated by condition of the animal. This experiment also demonstrates a practical method of colostrum feeding that could be followed on most dairy farms.

## EXPERIMENTAL PROCEDURE

Calves born in The Ohio State University dairy herd between November 30, 1945, and December 1, 1946, were divided into two groups at birth.

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The five major dairy breeds and both sexes were represented within the groups. The calves were not permitted to nurse. During the first 3 days they were nipple-pail fed colostrum from their dams at a rate of 10 per cent of their body weights. After 3 days, the control group (Group II) was fed Holstein milk at the following rates: 10 per cent of body weight for the first 3 months, 8 per cent of body weight during the fourth month, 6 per cent of body weight during the fifth month, and 4 per cent of body weight during the sixth month. The group receiving extra colostrum (Group I) was fed according to the same schedule except that part or all of the Holstein milk was replaced by colostrum when available. The term colostrum as used in this experiment refers to the production of the first 3 days immediately following parturition. No effort was made to store any of the colostrum and it was used completely at each milking. A concentrate mixture, mixed hay of average quality and water were provided *ad libitum* to all calves up to 3 months af age. After 3 months of age the calves were fed the concentrate mixture twice daily and the amount increased until each calf was receiving approximately 4 lb. daily at 6 months of age.

All calves were weighed at birth, on the third day, and at weekly intervals during the experimental period. Calves were bled for vitamin A and carotene analyses at the time of weighing during the first 4 weeks. After that, the calves were bled at the end of each succeeding 4-week period. Blood samples were drawn according to a definite schedule without consideration of the amount of colostrum fed during the interval between bleedings. Blood plasma analyses for vitamin A and carotenoid pigments were determined by the method of Kimble (7). An Evelyn photoelectric colorimeter with appropriate filters was used for each determination.

## EXPERIMENTAL RESULTS

A total of 76 calves, born in a 1-year period and made up of 19 Ayrshires, 17 Guernseys, 16 Jerseys, 15 Holsteins and 9 Brown Swiss, was used in this experiment. Twenty-two of the 36 calves starting the experiment in Group I were on experiment the full 24 weeks. Of the 40 calves in Group II at the beginning, 20 remained at the end of the 24-week period. The decline in numbers was primarily a result of selling bull calves and is without serious variation either in groups or breeds. Although a number of calves in both groups was afflicted with minor cases of scours, only 1 calf left the experiment as a result of calfhood diseases. This calf, a member of Group II, died of pneumonia.

During the experimental period 78 cows dropped calves and produced a total of 5,772 lb. of colostrum, or an average of 74.0 lb. per cow. Thirty per cent of the colostrum produced was used in feeding the calves during the 3-day period immediately following birth. The surplus, over 2 tons,

was used in feeding calves in Group I. Not all the colostrum used in this experiment was produced by the above cows, since colostrum feeding extended approximately 24 weeks beyond the time the last calf was allotted to the experiment. However, colostrum was produced during all seasons of the year and under both pasture and standard winter feeding conditiions.

Table 1 presents the data on the amount of colostrum fed per calf during the first 3 days and for each succeeding 4-week period. The number of calves included in each average is tabulated. There is a wide individual variation in the amounts of colostrum received by the calves in Group I. One calf received as much as 445 lb., while another in the same group received as little as 50 lb. The variation in amount fed per calf resulted from the variation in calving dates and the sale of calves.

Data on the sex distribution, average weekly gains, and cumulative weight gains by groups are presented in table 2. It will be noted that the calves of Group I made more rapid and consistent weight gains during the

TABLE 1

*Average amount of colostrum fed to each calf receiving extra colostrum for the first 3 days and by 4-week periods thereafter*

| | First 3 days[a] | 4-week period | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| Lb. of colostrum fed | 25.7 | 43.8 | 34.0 | 33.2 | 32.4 | 22.7 | 37.2 |
| Av. no. of calves | 36 | 35 | 32 | 26 | 25 | 19 | 17 |

[a] Each control calf received 25.2 lb. of colostrum during the first 3 days.

first 4 weeks and maintained that advantage throughout the experiment, even though the number of male calves is greater in the Group II. Another important observation was that the calves in Group I exhibited a more thrifty appearance as indicated by their alertness, quality and gloss of hair coat, and greater vigor. Although a regular weighing schedule was followed, the variation in weekly gains was to be expected because feed and water were given *ad libitum*.

The average data on vitamin A and carotene changes in the blood plasma for each group and for breeds within the groups are presented in tables 3 and 4. The blood plasma levels for both carotene and vitamin A are extremely low at birth. This is in agreement with previously reported experiments (10, 14). The substantial increases in the blood plasma vitamin A and carotene noted on the third day are attributed directly to colostrum feeding. The early peak levels of vitamin A reached on the third day were consistent for both groups. These levels were not exceeded in either group until the sixteenth week. In Group II the early peak level of carotene in the blood coincided with the early peak level for vitamin A, but in Group I the early peak level for carotene was not reached before the

second week. One should note the higher blood plasma levels of vitamin A and carotene found for Group I from the third to the sixteenth week as compared with those for Group II.

At 4 weeks of age, when the lowest blood plasma vitamin A and carotene values are observed, a significant difference between groups was found. At this age the residual effects of colostrum feeding for the first 3 days are at a minimum, and the low roughage consumption does not greatly

TABLE 2

*Sex distribution and average weight gains of calves in Groups I and II*

| Age of calf | Group I Extra colostrum | | | | Group II No extra colostrum | | | |
|---|---|---|---|---|---|---|---|---|
| | No. & sex of animals | | Av. weekly gain per calf | Av. cumulative gain of calves | No. & sex of animals | | Av. weekly gain per calf | Av. cumulative gain of calves |
| | M | F | | | M | F | | |
| (*wk.*) | | | (*lb.*) | (*lb.*) | | | (*lb.*) | (*lb.*) |
| 1 | 16 | 20 | 3.8 | 3.8 | 22 | 18 | 2.8 | 2.8 |
| 2 | 16 | 20 | 4.7 | 8.5 | 22 | 18 | 4.0 | 6.8 |
| 3 | 16 | 20 | 5.7 | 14.2 | 22 | 18 | 5.3 | 12.1 |
| 4 | 16 | 20 | 5.7 | 19.9 | 22 | 18 | 5.9 | 18.0 |
| 5 | 13 | 20 | 7.8 | 27.7 | 20 | 18 | 7.9 | 25.9 |
| 6 | 12 | 20 | 8.2 | 35.9 | 17 | 18 | 8.4 | 34.3 |
| 7 | 12 | 20 | 8.8 | 44.7 | 16 | 18 | 8.0 | 42.3 |
| 8 | 10 | 20 | 9.1 | 53.8 | 13 | 18 | 9.7 | 52.0 |
| 9 | 8 | 20 | 13.0 | 66.8 | 12 | 18 | 11.5 | 63.5 |
| 10 | 8 | 20 | 11.3 | 78.1 | 11 | 18 | 10.9 | 74.4 |
| 11 | 8 | 20 | 12.3 | 90.4 | 11 | 18 | 12.3 | 86.7 |
| 12 | 8 | 20 | 13.3 | 103.7 | 10 | 18 | 13.0 | 99.7 |
| 13 | 8 | 20 | 13.3 | 116.0 | 9 | 18 | 13.8 | 113.5 |
| 14 | 8 | 20 | 12.5 | 128.5 | 8 | 18 | 11.8 | 125.3 |
| 15 | 8 | 20 | 14.8 | 143.3 | 8 | 18 | 12.8 | 138.1 |
| 16 | 7 | 20 | 13.5 | 156.8 | 6 | 18 | 13.1 | 151.2 |
| 17 | 7 | 20 | 15.7 | 172.5 | 5 | 18 | 15.0 | 166.2 |
| 18 | 7 | 19 | 15.9 | 188.4 | 5 | 18 | 13.9 | 180.1 |
| 19 | 7 | 19 | 16.0 | 204.4 | 5 | 18 | 13.1 | 193.2 |
| 20 | 5 | 18 | 13.0 | 217.4 | 5 | 17 | 13.8 | 207.0 |
| 21 | 5 | 18 | 17.9 | 235.3 | 4 | 17 | 13.0 | 221.0 |
| 22 | 5 | 18 | 15.0 | 250.3 | 3 | 17 | 18.8 | 239.8 |
| 23 | 4 | 18 | 12.3 | 262.6 | 3 | 17 | 10.9 | 250.7 |
| 24 | 4 | 18 | 15.0 | 277.6 | 3 | 17 | 14.0 | 264.7 |

influence blood plasma carotene and vitamin A values. This significantly higher level of vitamin A must be attributed to the colostrum feeding. The higher blood plasma carotene noted after the eighth week can be attributed to the hay consumption.

It is to be noted that a breed difference occurs at birth in the blood plasma levels of vitamin A and carotene. The blood plasma of the Guernsey calves in this study was significantly lower in vitamin A and higher in carotene at birth than that of the other breeds. No significant seasonal variation in the blood plasma vitamin A was found.

TABLE 3

*Blood plasma vitamin A levels of calves used in the experiment*
*(Vitamin A expressed as γ per 100 ml.)*

| Group | Breed | Age in days | | | | Age in weeks | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | | 3 | | 1 | | 2 | | 3 | | 4 | | 8 | | 12 | | 16 | | 20 | | 24 | |
| | | No. | γ/ml. | No. | γ/ml. | No. | γ/ml. | No. | γ/ml. | No. | γ/ml. | No. | γ/ml. | No. | γ/ml. | No. | γ/ml. | No. | γ/ml. | No. | γ/ml. | No. | γ/ml. |
| I | Ayrshire | 7 | 5.6 | 7 | 17.5 | 7 | 15.7 | 6 | 14.6 | 5 | 16.2 | 5 | 12.7 | 5 | 14.6 | 4 | 18.3 | 4 | 18.4 | 3 | 19.1 | 3 | 21.4 |
| | Guernsey | 7 | 3.2 | 7 | 16.9 | 8 | 14.7 | 8 | 11.7 | 8 | 10.2 | 8 | 9.9 | 7 | 10.2 | 7 | 14.0 | 7 | 18.8 | 6 | 19.8 | 6 | 27.9 |
| | Holstein | 8 | 5.0 | 8 | 14.2 | 8 | 13.5 | 8 | 14.0 | 8 | 12.6 | 8 | 13.1 | 8 | 13.5 | 7 | 14.8 | 7 | 15.3 | 6 | 16.1 | 5 | 18.5 |
| | Jersey | 6 | 5.0 | 6 | 15.2 | 6 | 13.6 | 6 | 14.6 | 6 | 14.3 | 6 | 10.4 | 4 | 12.2 | 5 | 17.6 | 5 | 19.8 | 5 | 20.7 | 5 | 24.5 |
| | Brown Swiss | 5 | 5.4 | 5 | 17.9 | 5 | 14.7 | 5 | 17.4 | 5 | 16.1 | 5 | 13.2 | 5 | 12.2 | 5 | 16.3 | 5 | 16.4 | 3 | 21.2 | 3 | 21.9 |
| | Av. | 33 | 4.8 | 33 | 16.2 | 34 | 14.5 | 33 | 14.7 | 32 | 13.4 | 32 | 11.8 | 29 | 11.8 | 28 | 15.6 | 28 | 17.3 | 23 | 19.1 | 22 | 23.2 |
| II | Ayrshire | 8 | 5.8 | 10 | 20.4 | 11 | 16.1 | 11 | 15.7 | 10 | 11.9 | 11 | 11.9 | 8 | 12.3 | 7 | 15.6 | 4 | 19.9 | 5 | 23.2 | 4 | 26.1 |
| | Guernsey | 9 | 3.3 | 9 | 14.7 | 9 | 13.1 | 9 | 12.3 | 9 | 10.1 | 9 | 9.0 | 7 | 9.7 | 7 | 12.9 | 6 | 14.6 | 5 | 23.6 | 5 | 23.7 |
| | Holstein | 6 | 4.9 | 6 | 14.9 | 7 | 12.1 | 7 | 11.2 | 7 | 11.8 | 7 | 12.7 | 7 | 12.6 | 5 | 13.5 | 5 | 18.1 | 5 | 17.4 | 4 | 20.4 |
| | Jersey | 8 | 5.1 | 7 | 13.1 | 9 | 11.4 | 9 | 12.6 | 9 | 12.3 | 9 | 9.7 | 8 | 10.1 | 7 | 13.9 | 5 | 20.0 | 4 | 25.2 | 4 | 23.3 |
| | Brown Swiss | 3 | 6.6 | 4 | 15.7 | 4 | 14.9 | 4 | 14.6 | 4 | 14.5 | 3 | 11.2 | 4 | 11.5 | 4 | 13.1 | 4 | 14.5 | 3 | 14.5 | 3 | 20.9 |
| | Av. | 34 | 4.9 | 36 | 16.1 | 40 | 13.5 | 40 | 13.3 | 39 | 11.8 | 39 | 10.8 | 34 | 11.2 | 30 | 13.9 | 24 | 17.3 | 22 | 21.2 | 20 | 23.1 |

TABLE 4

*Blood plasma carotene levels of calves used in the experiment*
*(Carotene expressed as γ per 100 ml.)*

| Group | Breed | Age in days | | | | Age in weeks | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | | 3 | | 1 | | 2 | | 3 | | 4 | | 8 | | 12 | | 16 | | 20 | | 24 | |
| | | No. | γ/ml. | No. | γ/ml. | No. | γ/ml. | No. | γ/ml. | No. | γ/ml. | No. | γ/ml. | No. | γ/ml. | No. | γ/ml. | No. | γ/ml. | No. | γ/ml. | No. | γ/ml. |
| I | Ayrshire | 7 | 2.2 | 7 | 21.8 | 7 | 18.2 | 6 | 31.2 | 5 | 26.7 | 5 | 22.5 | 5 | 46.7 | 4 | 160.6 | 4 | 112.6 | 3 | 124.0 | 3 | 128.1 |
| | Guernsey | 8 | 5.0 | 7 | 65.1 | 8 | 72.2 | 8 | 73.2 | 8 | 61.0 | 8 | 60.0 | 7 | 104.2 | 7 | 162.4 | 7 | 253.4 | 6 | 235.6 | 6 | 267.5 |
| | Holstein | 8 | 3.5 | 8 | 25.4 | 8 | 32.2 | 8 | 40.4 | 8 | 30.1 | 8 | 29.1 | 8 | 32.2 | 7 | 55.0 | 7 | 76.0 | 6 | 101.2 | 5 | 141.5 |
| | Jersey | 5 | 3.7 | 6 | 32.1 | 6 | 33.9 | 6 | 41.6 | 6 | 40.5 | 6 | 25.2 | 4 | 41.4 | 5 | 122.8 | 5 | 212.8 | 5 | 200.5 | 5 | 171.5 |
| | Brown Swiss | 5 | 3.1 | 5 | 39.7 | 5 | 37.4 | 5 | 34.3 | 5 | 18.7 | 5 | 13.3 | 5 | 19.1 | 5 | 89.7 | 5 | 47.1 | 3 | 131.9 | 3 | 80.5 |
| | Av. | 33 | 3.6 | 33 | 36.4 | 34 | 39.7 | 33 | 46.0 | 32 | 37.4 | 32 | 32.6 | 29 | 51.1 | 28 | 115.3 | 28 | 144.8 | 23 | 164.9 | 22 | 172.5 |
| II | Ayrshire | 9 | 1.5 | 10 | 22.3 | 11 | 17.7 | 11 | 25.7 | 10 | 21.8 | 11 | 23.3 | 8 | 46.3 | 7 | 88.7 | 4 | 169.9 | 5 | 133.5 | 4 | 189.3 |
| | Guernsey | 9 | 4.8 | 9 | 75.2 | 9 | 66.9 | 9 | 72.4 | 9 | 60.6 | 9 | 65.7 | 7 | 109.1 | 7 | 152.8 | 6 | 213.8 | 5 | 267.8 | 5 | 337.6 |
| | Holstein | 6 | 2.3 | 6 | 25.6 | 7 | 28.4 | 7 | 29.9 | 7 | 22.6 | 7 | 18.8 | 7 | 39.5 | 5 | 54.4 | 5 | 86.8 | 5 | 84.7 | 4 | 145.7 |
| | Jersey | 8 | 4.0 | 7 | 38.1 | 9 | 34.7 | 9 | 26.1 | 9 | 26.9 | 9 | 17.7 | 8 | 70.8 | 7 | 193.1 | 4 | 194.9 | 4 | 318.0 | 4 | 267.2 |
| | Brown Swiss | 3 | 2.3 | 4 | 44.4 | 4 | 34.0 | 4 | 21.1 | 4 | 15.4 | 3 | 12.0 | 4 | 17.8 | 4 | 45.4 | 4 | 60.8 | 3 | 128.2 | 3 | 114.2 |
| | Av. | 35 | 3.1 | 36 | 41.6 | 40 | 36.1 | 40 | 36.5 | 39 | 31.4 | 39 | 30.8 | 34 | 60.3 | 30 | 119.7 | 23 | 148.6 | 22 | 185.6 | 20 | 224.1 |

TABLE 5

*The effect of the amount of extra colostrum fed on the blood plasma carotene and vitamin A and on the cumulative weight gains*

| Amount of colostrum fed | Day | | Week | | | | | | | | | Cumulated wt. gains |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 3 | 1 | 2 | 3 | 4 | 8 | 12 | 16 | 20 | 24 | |
| | *Vitamin A γ/100 ml.* | | | | | | | | | | | *(lb.)* |
| Over 200 lb. | 3.6 | 18.0 | 15.7 | 15.8 | 13.3 | 13.2 | 13.8 | 15.2 | 16.5 | 20.0 | 28.0 | 281.1 |
| Less than 200 lb. | 5.0 | 15.8 | 14.2 | 14.3 | 13.5 | 11.8 | 12.6 | 16.1 | 17.5 | 18.7 | 21.1 | 275.4 |
| No extra colostrum | 4.9 | 16.1 | 13.5 | 13.3 | 11.8 | 10.8 | 11.2 | 13.9 | 17.3 | 21.2 | 23.1 | 264.7 |
| | *Carotene γ/100 ml.* | | | | | | | | | | | |
| Over 200 lb. | 4.5 | 25.4 | 45.2 | 55.9 | 54.5 | 40.5 | 54.2 | 110.4 | 254.0 | 196.4 | 207.2 | |
| Less than 200 lb. | 3.2 | 38.9 | 38.4 | 43.4 | 32.7 | 30.4 | 50.1 | 116.9 | 108.5 | 151.1 | 156.4 | |
| No extra colostrum | 3.1 | 41.6 | 36.1 | 36.5 | 31.4 | 30.8 | 60.3 | 119.7 | 148.6 | 185.4 | 224.2 | |

Table 5 presents data showing the effects of feeding different amounts of colostrum on the blood plasma vitamin A, carotene, and cumulative weight gains of calves. It will be noted that the calves receiving more than 200 lb. of colostrum maintained higher levels of vitamin A in the blood plasma and made greater cumulative weight gains than those receiving less than 200 lb.

## DISCUSSION

Considerable research effort has been devoted to the development of calf rations, especially those limiting whole milk consumption. The past world war, with huge demands for food, pressed this development. Research workers (3, 9) have developed limited milk feeding schedules using rations fortified with known essential vitamins. In these experiments colostrum was fed only during the first 3 days and no attempts were made to utilize further the surplus colostrum produced. Wise and LaMaster (19) suggested the use of colostrum and reconstituted skim milk in the feeding of young calves. However, they questioned the advisability of feeding colostrum to older calves.

Responses to colostrum feeding, reflected by the blood plasma levels of vitamin A and carotene, substantiate earlier reports (10, 14). In the experiment herein reported, calves fed surplus colostrum, whenever available, maintained higher blood plasma levels for vitamin A and carotene, made more rapid weight gains and exhibited a more healthy appearance than did the control calves. These calves showed more luster to the hair coat and were more active and alert, especially during the first 2 months. Feeding of surplus colostrum did not prevent cases of scours. Such cases occurred in both groups and resulted in marked drops in the blood plasma vitamin A but did not materially affect the results. As in the previous experiment (14), no cases of scours could be attributed directly to colostrum feeding, even though on many occasions rations were changed abruptly from comptete milk rations to complete colostrum rations.

The economic importance of utilizing all colostrum in raising dairy calves is emphasized by this experiment. On the average, with five breeds and all ages of cows represented, the surplus colostrum produced amounted to 51.3 lb. per cow. In this experiment, with 78 cows represented, a total of 4,007 lb. of surplus colostrum was utilized in raising calves. If one-half as much surplus colostrum per cow from each of the 26 million dairy cows in the U.S.A. was utilized in calf raising, it would represent a saving of more than 650 million lb. of marketable milk. Allen (1) earlier recommended use of stored colostrum to replace marketable milk for raising dairy calves. The results of this experiment indicate that colostrum can be used to greatest advantage during the first month of life, when a calf must have milk in some form in its ration. During this period the calf needs special care and a well balanced ration if it is to survive and make thrifty

growth. After 4 to 6 weeks of age the feeding of extra colostrum has less marked effect because of greater consumption of hay and dry concentrate feeds. The consumption of these feeds, no doubt, also affects the rate of microbiological synthesis in the rumen.

## SUMMARY

Seventy-six calves, born in The Ohio State University dairy herd, were divided into comparable groups at birth. For the first 3 days all calves received colostrum from their dams. After 3 days, the calves in both groups were fed and managed similarly, except that for calves in Group I, colostrum, whenever available, replaced part or all of the regular ration of Holstein milk. The amounts of either milk or colostrum fed were determined by the body weights of the calves.

Calves in Group I maintained higher levels of blood plasma vitamin A and carotene, made more rapid weight gains, especially during the first 6 weeks, and exhibited a superior physical appearance. Abrupt changes in the amounts of colostrum fed, which in some instances varied from no colostrum to all colostrum, did not create any special management problems. The calves did not scour from colostrum feeding.

Complete utilization of all colostrum for calf feeding is important from an economic standpoint. Only 30 per cent of the colostrum produced in The Ohio State University dairy herd during the calendar year was used in feeding calves during the first 3 days. The balance, which exceeded 4,000 lb., was used to replace an equal amount of marketable milk in the feeding of calves in Group I. The general practice of using all the colostrum produced in the raising of calves would result in a substantial saving of marketable milk.

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# CAROTENE REQUIREMENTS FOR GUERNSEY AND JERSEY CALVES AS DETERMINED BY SPINAL FLUID PRESSURE

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Where calves of the various breeds are kept side by side, as in college herds, it has been noted that Guernsey and Jersey calves sometimes are more difficult to rear than Holstein or Ayrshire calves. The view has been expressed that this difference might be due to variations in carotene metabolism.

Boyer *et al.* (1) found that Guernsey calves required 57 γ of carotene per lb. of body weight to maintain a normal vitamin A level in the blood plasma, whereas Holstein calves required only 34 γ per lb. However, Nelson *et al.* (6) and Moore and Berry (4) did not note any difference in plasma vitamin A values from birth to 4 months of age between calves of the various dairy breeds, even when similar conditions of management were followed for all breeds. In these studies vitamin A as present in Holstein milk was fed to all the calves up to 2 months of age.

In order to gain further information on the question of whether there is a difference in the carotene requirements of the various breeds, the writers determined the carotene requirements of Guernsey and Jersey calves during the winter months, using cerebrospinal fluid pressure measurements as a criterion of adequacy.

The normal spinal fluid pressure varies between 75 to 120 mm. of water. Whenever the spinal fluid pressure exceeds 120 mm., it is considered abnormal and is taken as evidence that the calf is deficient in vitamin A. Previously published data (5) have shown that the spinal fluid pressure technique is quite precise and that it is possible to distinguish between differences in carotene intake of amounts as small as 2 γ per lb. of body weight. Where blood data are used, intakes must differ by as much as 10 to 15 γ per lb. before significant differences in blood values occur (5). In work on a considerable number of Holstein and Ayrshire calves, the authors never have observed an increase in spinal fluid pressure when 30 γ carotene per lb. were fed, whereas most calves on 28 γ show an elevated pressure. An increase above normal rather than the absolute value of the spinal fluid pressure is the deciding point in determining whether carotene intake is adequate.

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[1] The data for the Guernsey calves were collected while the senior author was located at the University of Maryland.

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## EXPERIMENTAL PROCEDURE

Four Guernsey male calves and four Jersey male calves were used in this experiment to determine the minimum amount of carotene necessary to prevent an increase in spinal fluid pressure. The four Guernsey calves were fed carotene as present in dehydrated alfalfa-leaf meal at the rate of 28, 32, 34, and 36 γ per lb. of body weight, respectively, while the four Jersey calves were fed at the rate of 28, 30, 32, and 34 γ, respectively.

These levels of intake were maintained throughout the winter months, and with one exception all calves were placed on their respective levels of intake during the early fall or winter months. There are some differences in ages of the calves used on the experiment. Previous experience has not demonstrated any marked differences in requirements of calves varying from 4 to 14 months of age. Methods and procedures were the same as previously outlined (5).

## RESULTS AND DISCUSSION

Table 1 shows the results for the Guernsey calves. The spinal fluid

TABLE 1

*Results obtained with four Guernsey male calves*

| Date | Age | Weight | Plasma vitamin A | Plasma carotene | Spinal fluid pressure |
|---|---|---|---|---|---|
| | *(days)* | *(lb.)* | *(γ/100 ml.)* | *(γ/100 ml.)* | *(mm. $H_2O$)* |
| | | *No. 472 (Intake 28 γ carotene/lb.)* | | | |
| 6–28–42 | 120 | 206 | 5.7 | 24 | ..... |
| 7–28–42 | 150 | 225 | 12.8 | 38 | 115 |
| 8–27–42 | 180 | 262 | 9.5 | 48 | 75 |
| 9–26–42 | 210 | 281 | 6.7 | 34 | 120 |
| 10–26–42 | 240 | 296 | 10.1 | 32 | ..... |
| 11–25–42 | 270 | 338 | 10.1 | 36 | 160 |
| 12–25–42 | 300 | 364 | 8.6 | 33 | 190 |
| 1–24–43 | 330 | 409 | 7.3 | 43 | 160 |
| | | *No. 496 (Intake 32 γ carotene/lb.)* | | | |
| 11–3–43 | 240 | 357 | 8.8 | 44 | ..... |
| 12–3–43 | 270 | 375 | 11.4 | 46 | 90 |
| 1–2–44 | 300 | 414 | 11.3 | 68 | 230 |
| | | *No. 496 (Intake increased to 40 γ carotene/lb.)* | | | |
| 2–1–44 | 330 | 456 | 14.1 | 71 | 160 |
| 3–2–44 | 360 | 487 | 12.8 | 67 | 90 |
| 4–1–44 | 390 | 542 | ..... | ..... | 90 |
| | | *No. 89 (Intake 34 γ carotene/lb.)* | | | |
| 11–15–44 | 120 | ..... | 9.1 | 19 | ..... |
| 12–15–44 | 150 | 182 | 13.8 | 39 | 90 |
| 1–14–45 | 180 | 209 | 10.9 | 54 | 100 |
| 2–13–45 | 210 | 236 | 10.9 | 67 | 100 |
| | | *No. 90 (Intake 36 γ carotene/lb.)* | | | |
| 12–15–44 | 120 | 181 | 15.0 | 29 | 90 |
| 1–14–45 | 150 | 206 | 12.5 | 49 | 100 |
| 2–13–45 | 180 | 241 | 10.4 | 45 | 90 |

pressure remained within normal limits throughout the experimental period for the two calves (nos. 89 and 90) that received 34 and 36 γ of carotene per lb. daily. At intakes of 28 and 32 γ (nos. 472 and 496) the spinal fluid pressure increased. When the carotene intake of no. 496 was increased to 40 γ per lb. after an increase in spinal fluid pressure had occurred, the spinal pressure values returned to normal in about 60 days. This effect may be partially seasonal, since unpublished data indicate that the cerebrospinal fluid pressure will start to decrease in March due to seasonal differences in carotene requirements. These data indicate that the minimum requirement for carotene by Guernsey calves is near 34 γ per lb. of body weight. The blood vitamin A values shown in this table also are of some interest. The vitamin A values for calf no. 496 during the period when his requirements were not being met (Nov. 3 to Jan. 2) were not noticeably lower than those for no. 89 or 90, and only in calf no. 472 were the vitamin A values below normal more or less consistently.

It will be noted that the spinal fluid pressure of calf no. 472 on the 28 γ level did not increase until he had been on experiment for about 4 months, whereas other calves showed increases in about 2 months. This calf was started during early summer and the delayed response is due to the fact that summer requirements (unpublished data) appear to be less than for the winter months.

Table 2 shows the results for the four Jersey calves. At levels of 32 and 34 γ of carotene per lb. of body weight, the spinal fluid pressure of calves no. 509 and 512 remained within normal limits (75 to 105 mm. $H_2O$). When the carotene intake of calves no. 2391 and 2392 was maintained at 28 and 30 γ, respectively, the spinal fluid pressure was above normal in both cases. Thirty-two micrograms of carotene per lb. of body weight therefore would appear to be the minimum requirement for Jersey calves, as compared with 34 γ per lb. for Guernsey calves. An examination of the plasma vitamin A values of these Jersey calves shows that these values were generally greater throughout the experimental period when carotene was fed at the highest level, but the differences in vitamin A values between calves no. 512 and 509, no. 509 and 2392, and no. 2392 and 2391 are so small that it is difficult to decide, on the basis of blood data alone, when the requirements actually were being met.

The requirement of 34 γ for Guernsey calves and 32 γ for Jersey calves is somewhat above the 30 γ level previously reported as being the minimum requirement for Holstein and Ayrshire calves. The differences in carotene requirement between the breeds therefore are not marked. In previous experiments with Holstein and Ayrshire calves there never has been an increase in spinal fluid pressure in calves fed as much as 30 γ or more of carotene per lb. of body weight. Yet in these experiments with Jersey and Guernsey calves, two receiving 30 and 32 γ showed elevated pressures. How-

ever, the 34 γ figure for Guernseys is much lower than that reported by Boyer *et al.* (1), whose data indicated a requirement of 57 γ of carotene per lb. of body weight for Guernsey calves and 34 for Holstein calves.

There are three possible explanations for this discrepancy. Boyer *et al.* (1) utilized blood plasma values as a criterion of adequacy. A study of the numerous data collected from this laboratory has shown that there

TABLE 2

*Results obtained with four Jersey male calves*

| Date | Age | Weight | Plasma vitamin A | Plasma carotene | Spinal fluid pressure |
|---|---|---|---|---|---|
| | *(days)* | *(lb.)* | *(γ/100 ml.)* | *(γ/100 ml.)* | *(mm. $H_2O$)* |
| | | *No. 2391 (Intake 28 γ carotene/lb.)* | | | |
| 8–29–46 | 200 | 285 | 5.7 | 39 | ..... |
| 9–28–46 | 230 | 339 | 5.5 | 56 | 95 |
| 10–28–46 | 260 | 364 | 6.5 | 55 | 170 |
| 11–27–46 | 290 | 415 | 7.8 | 58 | 165 |
| 12–27–46 | 320 | 448 | 7.9 | 89 | 185 |
| 1–26–47 | 350 | 465 | 9.7 | 102 | 175 |
| | | *No. 2392 (Intake 30 γ carotene/lb.)* | | | |
| 8–31–46 | 200 | 231 | 4.5 | 68 | ..... |
| 9–30–46 | 230 | 247 | 6.8 | 94 | 135 |
| 10–30–46 | 260 | 294 | 7.8 | 106 | 160 |
| 11–29–46 | 290 | 321 | 9.0 | 99 | 195 |
| 12–29–46 | 320 | 342 | 9.1 | 106 | 165 |
| 1–28–47 | 350 | 396 | 8.6 | 110 | 210 |
| | | *No. 509 (Intake 32 γ carotene/lb.)* | | | |
| 8–27–46 | 190 | 247 | 6.4 | 83 | ..... |
| 9–26–46 | 220 | 275 | 6.2 | 107 | 75 |
| 10–26–46 | 250 | 321 | 8.1 | 134 | 80 |
| 11–25–46 | 280 | 402 | 9.2 | 138 | 75 |
| 12–25–46 | 310 | 431 | 11.9 | 175 | 75 |
| 1–24–47 | 340 | 440 | 10.0 | 146 | 80 |
| | | *No. 512 (Intake 34 γ carotene/lb.)* | | | |
| 8–31–46 | 170 | 173 | 7.2 | 42 | ..... |
| 9–30–46 | 200 | 213 | 8.0 | 51 | 100 |
| 10–30–46 | 230 | 237 | 8.8 | 77 | 90 |
| 11–29–46 | 260 | 300 | 10.6 | 62 | ..... |
| 12–29–46 | 290 | 327 | 10.8 | 110 | 105 |
| 1–28–46 | 320 | 378 | 12.7 | 103 | 85 |

must be a spread in intake of at least 10 to 15 γ of carotene per lb. of body weight before a good correlation with blood data can be noted. The blood data in tables 1 and 2 further emphasize this point. Calves on the same intake show considerable individual variation in plasma vitamin A values. There also may be differences in requirements between various strains or families of Guernsey cattle. If inheritance plays some part in causing higher carotene requirements for Guernsey than for Holstein

calves, it is probable that there would be differences in families or strains of Guernseys. This point should be investigated. Another possible cause for the difference between the results from this laboratory and those from Wisconsin might be the difference in some environmental factor. Unpublished data show a higher requirement for the winter months than for the summer months.

The vitamin A content of the blood plasma of the Guernsey and Jersey calves used in this experiment is of the same order as that of the Holstein and Ayrshire calves that were fed at similar levels of carotene intake in previous experiments (5). This would indicate further that the carotene requirement of the Guernsey and Jersey calves used in this experiment was not greatly different from that of the Holstein and Ayrshire calves. Even though the difference is not great, it might account for some of the difficulty that sometimes is encountered in raising Guernsey calves, particularly if the hay quality is very poor or hay consumption is not adequate.

Requirements reported in this experiment are about double those reported by Moore (3) and Guilbert *et al.* (2), where night blindness was used as a criterion. The latter authors term their requirements "physiological minimum". In the light of the present and previous data (5), such an interpretation no longer is tenable. Furthermore, minimum requirements should be based on the least determinable change produced in the animal by a deficiency of the nutrient under study. The carotene requirements for calves as determined by cerebrospinal fluid pressure would appear to meet this definition more closely than night blindness, since changes in spinal fluid pressure occur before night blindness can be detected. The requirements as determined by the night blindness technique therefore cannot be considered adequate.

## SUMMARY

1. Guernsey calves under our experimental conditions required an intake of 34 $\gamma$ of carotene per pound of body weight during the winter months to maintain a normal spinal fluid pressure. On the same basis, Jersey calves required an intake of 32 $\gamma$. If inheritance is found to affect carotene requirements, it is possible that these values might need to be modified even for strains within the breed.

2. This requirement is slightly higher than for Holstein and Ayrshire calves, which, under similar conditions, require 30 $\gamma$ of carotene per lb. of body weight.

3. These results appear to indicate that somewhat more attention should be given to the quality of hay used in feeding Guernsey and Jersey calves than in feeding Holstein or Ayrshire calves.

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# MANUFACTURE OF POWDERED CREAM FOR WHIPPING BY AERATION[1]

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A number of contributions were made during the war to the knowledge of methods for the successful manufacture of powdered whole milk and ice cream mix. Because of the success of industry with these two items, it is logical to consider the application of dehydration to certain other types of dairy products, such as cream. In 1922 Babcock (2) demonstrated that reconstituted powdered cream containing as much as 40 per cent fat failed to whip. He concluded that such a product may be considered as useless for whipping purposes.

In 1937 Getz *et al.* (3) reported on a method for whipping cream by aeration. The cream is charged with nitrous oxide at a pressure of about 175 lb. in a specially constructed steel container. The cream under pressure is released to the atmosphere through a Schrader valve,[2] resulting in a greatly expanded volume. While this method involves gasification of the serum portion of the cream, air incorporation in ordinary cream whipping is dependent upon a partial clumping of the fat. Because of this fundamental difference, it was thought that the process of drying cream to be whipped by aeration would have little or no effect upon either the gas dispersion or the stiffness of the whip. This study was made to test this hypothesis.

## EXPERIMENTAL PROCEDURE

The cream-mix was made from fresh sweet cream, condensed skim milk, skim milk, sugar, stabilizer and flavoring. It then was pasteurized at temperatures not over 160° F. for 30 minutes and spray dried. Attempts were made to keep the iron and copper content at a minimum. The importance of each of the following factors was studied: (a) composition of the cream-mix; (b) nozzle size; (c) spray pressure; and (d) relation of inert gas and certain antioxidants to keeping quality.

Some of the experiments reported herein were conducted during World War II with a product containing 19 per cent butterfat on a reconstituted basis. Unless otherwise stated, the remainder of the experiments have been conducted with mix containing approximately 30 per cent butterfat on a

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[1] Process and container patented by Aeration Processes, Inc., Columbus, Ohio (U. S. Patent nos. 2,294,172 and 2,281,604).

[2] The Schrader valve originally employed has been superseded by an entirely new valve of original design (Model F2). This newly constructed valve meets the specifications of the U. S. Public Health Service.

reconstituted basis. Sucrose was used as the sweetening agent and was added before drying. Pure vanilla concentrate was used for flavoring the cream. The cream-mix was not condensed before drying, as the total solids content made this procedure unnecessary. All the cream-mixes were dried on an experimental spray drier using a no. 72 nozzle with a no. 20 core unless otherwise stated.

In the keeping quality studies, the powdered cream-mix was uniformly mixed and packed in no. 1 tin cans, the same weight of dried cream-mix being put into each can. After sealing, the cans were gas packed where necessary for the experiment.

For whipping purposes the dried cream-mix was reconstituted in the proper ratio with cold tap water and held for 24 hours or more at 40° F. Eight ounces of cream-mix were placed in Instantwhip[3] containers, which then were charged with nitrous oxide gas and chilled before dispensing. The dispensed product usually was examined for drainage, overrun, flavor, body and appearance. Drainage was measured by dispensing an Instantwhip container into a 6-inch funnel, fitted with a screen at the apex, and set in a graduated cylinder (fig. 1). The volume (ml.) of drainage secured in 30 minutes at room temperature was recorded as the measurement of the amount of drainage.

On the powdered samples held in storage, changes in oxygen concentration in the headspace gas and changes in palatability were obtained at various intervals. The oxygen values were secured by the Manometric procedure of Van Slyke and Sendroy (12) and the flavor scores by two or more judges using the ice cream score card based on a flavor score of 45 out of a possible 50 points.

Moisture was determined by the toluol distillation method (1), butterfat of the powdered cream-mix by the Mojonnier method (8), copper by the procedure of Hetrick and Tracy (5), and iron by the method of Pyenson and Tracy (9).

## RESULTS

*Effect of variations of butterfat on whipping properties and appearance of reconstituted powdered cream-mix.* Cream-mixes containing 26, 30, 32 and 34 per cent butterfat were prepared (batches no. 1, 2, 3 and 4, respectively). The milk solids-not-fat were standardized to 7.5 per cent Other ingredients added were 5 per cent sugar, 0.3 per cent emulsifying agent and 0.1 per cent vanilla. After drying, a portion of each of the four lots was reconstituted with the proper amount of water, aged for 24 hours, and then placed in the containers, gassed and chilled before dispensing. The samples were shaken uniformly and dispensed simultaneously. There was only 1.5 ml. drainage from the 26 per cent product, 0.5 ml. drainage

[3] "Instantwhip" is the trade name for the product as distributed by Aeration Processes, Inc., Columbus, Ohio.

from the 30 and 32 per cent products and no drainage from the 34 per cent product. The flavors of all the samples were identical. As the fat content was increased from 26 to 34 per cent, an improvement in the body and texture resulted and the product became drier in appearance. Since only a slight advantage resulted from using 32 or 34 per cent fat cream-mix as compared with the 30 per cent fat cream-mix, it was decided that future trials with heavy cream-mix would be limited to mixes containing 30 per cent butterfat.

Another factor that influenced the decision to standardize on 30 per cent butterfat was the heavy viscosity obtained with the higher-testing samples. Reconstituted cream-mix made from dried cream has a greater viscosity than the original cream-mix before drying. Too great a viscosity interferes with filling and the containers must be shaken longer to dissolve the nitrous oxide gas in the cream.

*Effect of variations in the milk solids-not-fat on whipping properties and appearance of reconstituted powdered cream-mix.* The mixes for this study were standardized to contain 30 per cent butterfat, 5 per cent sugar, 0.3 per cent emulsifying agent and 0.1 per cent vanilla. The variations in the milk solids-not-fat were 6, 7, 8 and 9 per cent (batches no. 5, 6, 7 and 8, respectively). Concentrated skim milk was used to increase the percentage of non-fat milk solids.

On reconstitution, the sample containing the 9 per cent milk solids-not-fat had the greatest viscosity, while the sample with the normal milk solids-not-fat (6 per cent) had the least viscosity. Increasing the milk solids-not-fat produced a heavier body in the whipped cream-mix. The flavor and standing-up qualities of the whipped cream-mix also were improved by the additional milk solids-not-fat.

Drainage from the whipped cream-mix was reduced by increasing the milk solids-not-fat content of the cream-mix. However, 9 per cent milk solids-not-fat in the 30 per cent butterfat cream-mix reduced overrun and produced a moist body. Since too great a viscosity produces mechanical difficulties in filling the containers and since too moist an appearance is produced by more than approximately 8 per cent milk solids-not-fat, it was deemed advisable to limit the milk solids-not-fat content to approximately 8 per cent in 30 per cent butterfat cream-mix.

*Comparison of whipped reconstituted powdered cream-mix made with and without added emulsifying agent.* These comparisons were made using several emulsifiers. Typical results of a representative emulsifying agent are presented. Cream-mixes containing 29.5 per cent butterfat, 7.5 per cent milk solids-not-fat, and 5 per cent sugar were prepared without and with 0.2 per cent glycerol monostearate. These mixes (batches 9 and 10) were dried and reconstituted in the usual manner. There was no drainage at room temperature in either sample in 0.5 hour. The flavors of both

samples were identical. The sample containing the emulsifying agent whipped to a higher overrun at any given pressure and the whipped cream had a drier appearance, a better body and a finer texture than the control sample containing no emulsifier. Commercial products containing emulsifying agents such as sorbitan monostearate also were found to be satisfactory. Gelatin and sodium alginate stabilizers alone were not satisfactory for powdered cream-mix for whipping purposes. They produced a moist appearance in the whipped cream.

*Effect of nozzle size on whipping properties of reconstituted cream-mixes.* A mix containing 29 per cent butterfat, 7 per cent milk solids-not-fat, 5 per cent sugar and 0.3 per cent emulsifying agent was used. The nozzles used were nos. 79, 72 and 65, representing openings of 0.0145, 0.025 and 0.035 inch, respectively (batches 11, 12 and 13).

There were no significant differences in whipping properties, body and texture or appearance of whipped reconstituted cream-mixes sprayed with the different nozzles. The capacity of the drier was lowered by using nozzles with smaller orifices. The packing density was increased by using nozzles with relatively large orifices.

*Relation of spray pressure to the whipping properties of reconstituted powdered cream-mix.* Homogenization is detrimental to the body, texture, appearance and drainage of cream whipped by aeration. The lower the fat content of the cream, the more detrimental is the effect of homogenization (10).

To determine whether the spraying process has any detrimental effect on the whipping properties of reconstituted powdered cream-mix, a lot of cream-mix (19 per cent butterfat, 9 per cent milk solids-not-fat, 6 per cent sugar, 0.15 per cent gelatin, and 0.2 per cent emulsifying agent) was divided into three portions and sprayed at: (a) 200 lb. pressure, (b) 1,500 lb. pressure, and (c) 2,800 lb. pressure (batches 14, 15 and 16).

The amount of spray pressure used had no significant effect on the overrun obtained on powdered cream-mix whipped by aeration (table 1). The drainage, however, was nearly doubled as the pressure was increased from 200 to 2,800 lb. As the spray pressure was increased, the whipped cream-mix became more moist in appearance and contained larger gas cells. Similar results were obtained with 30 per cent reconstituted powdered cream-mix, except that the detrimental effect of the higher spray pressures was not as pronounced as with 19 per cent cream-mix.

*Relation of inert gas and certain antioxidants to keeping quality.* In the commercial manufacture of powdered whole milk, replacing air in the package with nitrogen and/or carbon dioxide has been found to decrease the intensity of the oxidized flavor over a period of time and, in some cases, delay onset of oxidized flavor development. In preliminary trials it was shown that dried cream-mix packed in air could be held only a few weeks

at room temperature before a stale or oxidized flavor developed. To have commercial value, it is necessary that dried cream-mix keep for longer periods of time. That the oxidized flavor can be delayed by the addition of certain antioxidants to milk before drying has been demonstrated by Hollender and Tracy (6), Tracy *et al.* (11), Jack and Henderson (7), Waite (13), and Hetrick and Tracy (5).

To determine whether the use of antioxidants would prolong the keeping quality of dried cream-mix, six 50-lb. lots of cream-mix (29.5 per cent butterfat, 8 per cent milk solids-not-fat, 5 per cent sugar and 0.2 per cent glycerol monostearate) were dried, containing the following levels of antioxidants. The indicated percentages represent the amounts used as calculated on the basis of the weight of the fat: Batch no. 17, control, no added antioxidants; 18, Viobin antioxidant, 0.1 per cent; 19, nordihydroguaiaretic acid (NDGA), 0.03 per cent; 20, gallic acid, 0.1 per cent; 21, sodium

TABLE 1

*Effect of spray pressure on whipping properties of reconstituted powdered cream-mix*

| Batch no. | Spray pressure | Overrun | Drainage | Firmness | Gas cell structure | Dryness |
|---|---|---|---|---|---|---|
| | (*lb.*) | (%) | (*ml.*) | | | |
| 14 | 200 | 500 | 22 | Good | Small uniform gas cells | Dry |
| 15 | 1,500 | 490 | 39 | Poor | Large irregular gas cells | Moist |
| 16 | 2,800 | 510 | 43 | Very poor | Large irregular gas cells | Moist |

arabo ascorbate, 0.1 per cent; and 22, natural mixed tocopherols, 0.1 per cent.

All of the antioxidants were added directly to the cream-mix at the preheater just before spray drying, except the NDGA, which was dissolved in 15 mm. of butter oil before adding to the preheater. Both air- and nitrogen-packed samples were prepared. The samples were stored at room temperature. Data taken during 6 months of storage are given in table 2.

Oxygen concentration in the headspace gas gradually was lowered and the dried cream became less palatable as the storage period advanced. A stale flavor usually preceded the oxidized flavor in both air and nitrogen packed samples. After 180 days the nitrogen-packed control sample, although slightly oxidized, was still palatable, while the air-packed control sample had a pronounced oxidized flavor.

At the end of 6 months of storage at room temperature, all air-packed batches containing antioxidants were oxidized except batch no. 22, which had become intensely metallic[4] after 5 weeks of storage. Through the 121-day storage period, the air-packed batches containing antioxidants scored

[4] Not to be confused with the typical oxidized or tallowy flavor.

consistently higher than the control that contained no antioxidant. The addition of antioxidants to powdered cream-mix, especially when nitrogen packed, increased the keeping quality of the powder. The most effective antioxidants were NDGA, gallic acid and Viobin. The sodium arabo ascorbate delayed onset of the oxidized flavor but produced a cooked or "nutty" flavor in the cream-mix, which persisted throughout the storage period. Samples containing mixed tocopherols developed a very metallic off-flavor after 5 weeks of storage.

TABLE 2

*Changes in oxygen concentration in head space gas and palatability of air-packed and gas-packed dried cream containing antioxidants*

| Batch no. | Antioxidant | | Days storage at room temperature | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 7 | 14 | 35 | 58 | 121 | 180 |
| 17 | Control | % oxygen | 20.46 | ..... | 20.62 | 20.14 | 19.78 | 18.79 |
| | | Flavor | 45 | ..... | 41 | 39 | 38.5[b] | 37.5 |
| 17N[a] | Control | % oxygen | 0.85 | 1.06 | 0.84 | 0.68 | 0.62 | 0.28 |
| | | Flavor | 45 | ..... | 42 | 41[b] | 40.5 | 40 |
| 18 | Viobin | % oxygen | 20.11 | ..... | 20.56 | 20.56 | 19.77 | 19.22 |
| | | Flavor | 45 | ..... | 43 | 41 | 40 | 39.5[b] |
| 18N | Viobin | % oxygen | 1.03 | 0.76 | 1.11 | 0.47 | 0.52 | 0.51 |
| | | Flavor | 45 | ..... | 43.5 | 42 | 42 | 42 |
| 19 | NDGA | % oxygen | 20.0 | ..... | 20.12 | 20.44 | 20.11 | 19.84 |
| | | Flavor | 45 | ..... | 43.5 | 43 | 43 | 41[b] |
| 19N | NDGA | % oxygen | 1.08 | 1.02 | 0.95 | 1.03 | 0.19 | 0.33 |
| | | Flavor | 45 | ..... | 44 | 43 | 43 | 42.5 |
| 20 | Gallic acid | % oxygen | 20.04 | ..... | 20.35 | 20.53 | 20.07 | 18.11 |
| | | Flavor | 45 | ..... | 44 | 43 | 43 | 40[b] |
| 20N | Gallic acid | % oxygen | 0.92 | 0.50 | 0.51 | 0.54 | 0.45 | 0.21 |
| | | Flavor | 45 | ..... | 44.5 | 43.5 | 43.5 | 42 |
| 21 | Sodium arabo ascorbate | % oxygen | 20.35 | ..... | 20.3 | 19.91 | 19.64 | 18.26 |
| | | Flavor | 42.5 | ..... | 42.5 | 42 | 41 | 40[b] |
| 21N | Sodium arabo ascorbate | % oxygen | 1.06 | 0.71 | 0.76 | 0.65 | 0.56 | 0.00 |
| | | Flavor | 42.5 | ..... | 42.5 | 42.5 | 42.5 | 42 |
| 22 | Natural mixed tocopherols | % oxygen | 20.39 | ..... | 20.35 | 20.09 | 16.29 | 18.32 |
| | | Flavor | 41 | ..... | 35[c] | 33[c] | 30[c] | 25[c] |
| 22N | Natural mixed tocopherols | % oxygen | 0.92 | 0.41 | 0.89 | 0.53 | 0.19 | 0.27 |
| | | Flavor | 41 | ..... | 35[c] | 33[c] | 30[c] | 25[c] |

[a] N indicates samples were nitrogen packed. Others were air packed.
[b] Time when oxidized flavor first was noticed.
[c] Metallic—not to be confused with oxidized or tallowy.

*Vanilla as an antioxidant.* In another study that was repeated three times with practically the same results, powdered cream-mixes containing NDGA and a pure 6-fold vanilla concentrate (made from Bourbon and Mexican beans) were compared as to keeping properties. The cream-mix had a composition of 30 per cent butterfat, 7.35 per cent milk solids-not-fat, 5 per cent sugar, and 0.2 per cent glycerol monostearate. The NDGA was added at two levels, 0.0025 per cent and 0.005 per cent, based on the

TABLE 3

*Changes in oxygen concentration in head space gas and palatability of air-packed and gas-packed dried cream-mix containing antioxidants*

| Batch no. | Antioxidant | | Days storage at room temperature | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 7 | 30 | 60 | 92 | 120 | 152 | 182 | 213 | 265 | 365 |
| 23 | Control | % oxygen | 20.61 | 20.05 | 19.40 | 19.16 | 18.44 | 17.14 | 17.11 | 15.95 | 12.45 | 2.12 |
| | | Flavor | 41.5 | 38.0[b] | 36.0 | 35.0 | 33.0 | 33.0 | 33.0 | 31.0 | 20.0 | 20.0 |
| 23N[a] | Control | % oxygen | 1.66 | 1.63 | 1.11 | 1.37 | 0.77 | 0.30 | 0.65 | 0.52 | 0.89 | 0.21 |
| | | Flavor | 43.0 | 39.0[b] | 39.0 | 38.0 | 41.0 | 40.5 | 39.5 | 38.0 | 36.0 | 35.0 |
| 24 | 0.0025%[c] NDGA | % oxygen | 20.41 | 20.19 | 19.74 | 19.58 | 19.71 | 19.07 | 17.16 | 18.56 | 15.76 | 12.70 |
| | | Flavor | 43.0 | 41.5[b] | 39.0 | 39.0 | 39.0 | 38.5 | 35.0 | 33.0 | 34.0 | 30.0 |
| 24N | 0.0025% NDGA | % oxygen | 1.65 | 1.89 | 1.35 | 1.65 | 1.37 | 0.91 | 0.47 | 1.00 | 0.52 | 0.32 |
| | | Flavor | 43.0 | 42.5 | 43.0 | 40.0[b] | 41.0 | 41.0 | 40.0 | 39.0 | 38.0 | 37.0 |
| 25 | 0.005%[c] NDGA | % oxygen | 20.71 | 20.34 | 20.19 | 20.04 | 17.98 | 18.61 | 17.13 | 17.02 | 18.02 | 7.26 |
| | | Flavor | 44.0 | 42.0 | 42.0 | 42.0 | 38.0[b] | 39.0 | 35.0 | 34.0 | 38.0 | 34.0 |
| 25N | 0.005% NDGA | % oxygen | 2.22 | 2.22 | 1.45 | 2.05 | 1.11 | 0.72 | 1.23 | 1.17 | 0.49 | 0.15 |
| | | Flavor | 44.0 | 44.0 | 43.0 | 42.0 | 40.0[b] | 41.5 | 40.5 | 39.5 | 40.0 | 37.0 |
| 26 | 0.1% pure vanilla conc. | % oxygen | 21.04 | 20.41 | 19.96 | 19.93 | 19.71 | 19.13 | 18.69 | 18.40 | 15.54 | 11.58 |
| | | Flavor | 44.0 | 44.0 | 44.0 | 43.0 | 42.0 | 41.0[b] | 37.0 | 37.0 | 39.0 | 34.0 |
| 26N | 0.1% pure vanilla conc. | % oxygen | 2.13 | 2.40 | 2.04 | 2.16 | 1.88 | 1.51 | 1.42 | 2.13 | 1.11 | 1.23 |
| | | Flavor | 44.0 | 44.0 | 44.0 | 43.5 | 43.0 | 42.5 | 41.0[b] | 41.0 | 41.0 | 40.0 |

[a] N indicates samples were nitrogen packed. Others were air packed.
[b] Time when oxidized flavor first was noticed.
[c] Based on per cent of fat.

weight of butterfat. It was added in 15 ml. of butter oil to the 60 per cent cream-mixes just before drying. To another mix, 0.1 per cent of pure vanilla extract was added just before drying. The data obtained during a year's storage at room temperature are given in table 3.

The antioxygenic properties of the vanilla were discovered when it was added to the cream-mix before drying to note whether the vanilla flavor was lost in the drying operation. This vanilla concentrate was added

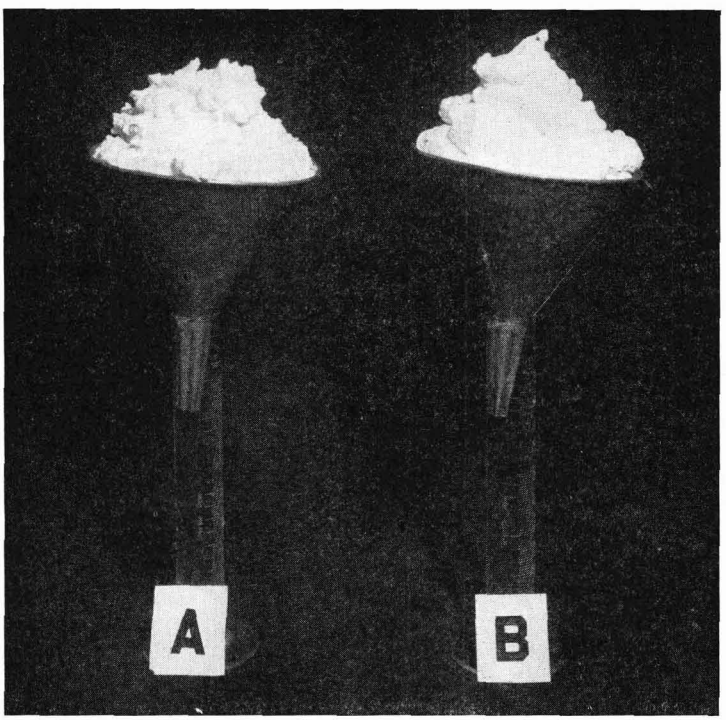


FIG. 1. Method used to measure drainage from whipped cream-mix. (A = Aerated cream made from fresh cream-mix (29.5% butterfat) B = Aerated cream made from reconstituted powdered cream-mix (29.5% butterfat). Both products made from same lot of cream.)

at the preheater just before spray drying the cream-mix at a temperature of 150° F. The processing or the drying operations did not seem to affect the intensity of the vanilla flavor of the reconstituted powdered cream-mix. The data indicate that this pure vanilla concentrate was a more effective antioxidant than NDGA. The nitrogen-packed control sample was oxidized at the 30-day examination period. The sample that contained 0.0025 per cent of NDGA was oxidized at 92 days of storage; when 0.005 per cent NDGA was added, the powdered cream-mix was oxidized at the

120-day examination. Nitrogen-packed powdered cream-mix containing 0.1 per cent of pure vanilla concentrate was not oxidized until examined at 182 days of storage, and then it was only slightly oxidized. Air-packed samples showed similar trends when stored with NDGA, and pure vanilla concentrate. At first the judges thought that the vanilla might be masking the oxidized flavor in the samples containing the vanilla. To check this possible masking, 0.1 per cent pure vanilla concentrate was added to a reconstituted control that was oxidized. The results obtained indicate

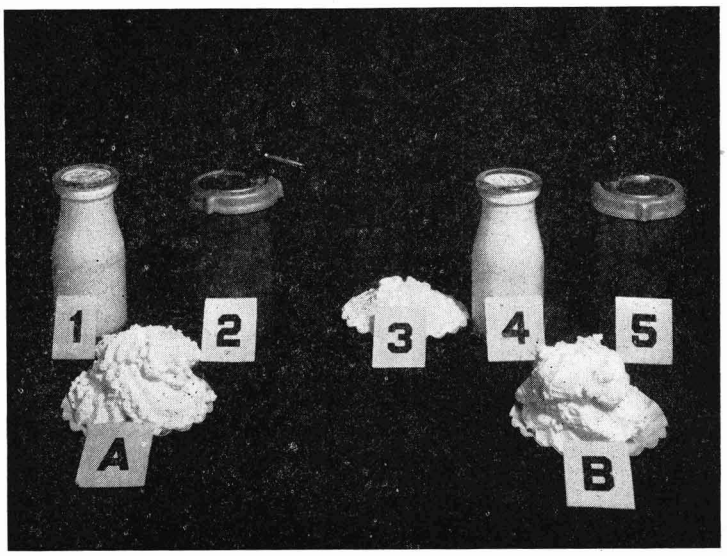


FIG. 2. Comparison of aerated cream made from fresh and reconstituted cream-mix (29.5% butterfat) (1 = Fresh cream-mix; 2 = charged container of fresh cream-mix; 3 = powdered cream-mix; 4 = reconstituted powdered cream-mix; 5 = charged container of reconstituted cream-mix; A = Aerated cream made from fresh cream-mix; B = Aerated cream made from reconstituted cream-mix. Both products made from same lot of cream).

that there was little, if any, masking of the oxidized flavor by the vanilla flavor.

The gas analysis data in table 3 indicate that when the vanilla concentrate was used, in general, there was more oxygen left in the headspace than in the headspace of the control or NDGA samples at the end of the storage period.

The chief constituent of vanilla beans from which vanilla extract is made is vanillin. Vanillin is a phenolic substance having the formula 4-hydroxy 3-methoxy benzaldehyde. Vanillin is the mono-methyl ether of protocatetheric aldehyde, the methoxy group being in the meta position to the aldehyde group. At low concentrations numerous phenolic substances

have the ability markedly to inhibit the autoxidation of fats. The most effective phenols are those which have some type of oxygen linkage in the ortho or para positions, or both, to the hydroxyl group. Some of the best known antioxidants of this type are hydroquinone, the tocopherols, gum guaiac and NDGA. The structural formula of vanillin is quite similar to other compounds that show antioxygenic properties. Consequently, it is possible the antioxidant properties of certain vanillas can be explained through their structural formulas.

*Comparison of fresh cream-mix with reconstituted powdered cream-mix.* To determine whether or not aerated cream made from powdered cream-mix is as satisfactory as aerated cream made from fresh cream-mix, a batch of cream-mix containing 29.5 per cent fat was divided into two lots. One lot was kept as fresh cream-mix. The other lot was dried in the usual manner and reconstituted with water to bring it back to its original composition of 29.5 per cent butterfat, 8 per cent milk solids-not-fat, 5 per cent sugar and 0.3 per cent emulsifying agent. Batch no. 27A, made from the fresh cream-mix, is exhibit A in figures 1 and 2 and batch no. 27, made from the reconstituted cream-mix, is exhibit B.

TABLE 4

*Butterfat, moisture, iron and copper content of powdered cream-mixes*

| Batch no. | Butterfat | Total solids | Moisture | Iron | Copper |
|---|---|---|---|---|---|
| | (%) | (%) | (%) | (*p.p.m.*) | (*p.p.m.*) |
| 1 | 66.25 | 99.5 | 0.5 | 4.2 | 1.18 |
| 2 | 69.06 | 99.4 | 0.6 | 2.4 | 1.23 |
| 3 | 70.25 | 99.0 | 1.0 | 2.4 | 0.90 |
| 4 | 72.09 | 99.6 | 0.4 | 2.6 | 0.78 |
| 5 | 72.04 | 99.4 | 0.6 | 3.8 | 1.50 |
| 6 | 69.91 | 99.5 | 0.5 | 3.2 | 1.25 |
| 7 | 69.09 | 99.4 | 0.6 | 3.1 | 1.43 |
| 8 | 67.03 | 99.6 | 0.4 | 2.5 | 1.40 |
| 9 | 68.40 | 99.4 | 0.6 | 3.6 | 0.85 |
| 10 | 68.76 | 99.3 | 0.7 | 3.5 | 1.23 |
| 11 | 68.20 | 99.5 | 0.5 | 2.2 | 1.35 |
| 12 | 68.31 | 99.6 | 0.4 | 2.0 | 1.00 |
| 13 | 68.16 | 99.5 | 0.5 | 1.9 | 0.95 |
| 14 | 55.19 | 98.9 | 1.1 | 4.2 | 1.00 |
| 15 | 55.19 | 98.7 | 1.3 | 2.6 | 1.35 |
| 16 | 55.20 | 98.9 | 1.1 | 2.3 | 1.20 |
| 17 | 68.42 | 99.4 | 0.6 | 3.1 | 1.30 |
| 18 | 67.89 | 99.4 | 0.6 | 3.2 | 1.23 |
| 19 | 67.67 | 99.2 | 0.8 | 2.7 | 1.18 |
| 20 | 67.79 | 99.4 | 0.6 | 3.7 | 1.18 |
| 21 | 67.76 | 99.2 | 0.8 | 3.7 | 1.20 |
| 22 | 67.93 | 99.2 | 0.8 | 2.5 | 1.15 |
| 23 | 69.92 | 99.2 | 0.8 | 3.0 | 0.85 |
| 24 | 69.87 | 99.1 | 0.9 | 2.2 | 0.75 |
| 25 | 69.97 | 99.4 | 0.6 | 1.4 | 0.63 |
| 26 | 69.89 | 99.2 | 0.8 | 1.8 | 0.83 |
| 27 | 67.21 | 98.9 | 1.1 | 2.8 | 1.40 |

The only differences between the aerated cream-mix and the powdered

cream-mix were the dryness of the whip and the cooked flavor. The body and texture of the fresh product was firm and dry while the reconstituted product was firm but slightly moist. The flavor of the reconstituted product was slightly cooked.

*Composition of powdered cream-mixes.* Butterfat, moisture and iron and copper content were determined on all of the batches reported in this paper. Table 4 gives a summary of these data. The butterfat percentages of the dried cream-mixes recorded in the table vary somewhat due to variations in composition of the cream-mixes before drying. Batches 14, 15 and 16 were made with 19 per cent cream-mix. All other batches contained 29–30 per cent fat except batches 1, 2, 3 and 4, in which the butterfat was varied from 26 to 34 per cent. No difficulty was encountered in producing a cream-mix with low moisture content, since the dried product consisted of almost 70 per cent butterfat. The iron and copper contents of the dried cream-mixes are similar to the iron and copper contents of powdered whole milk made with the equipment used.

## SUMMARY

Studies were made of variations in percentage of butterfat, milk solids-not-fat, and emulsifying agents in powdered cream-mixes. A product consisting of approximately 30 per cent butterfat, 7 to 8 per cent milk solids-not-fat, 5 per cent sugar, 0.3 per cent emulsifying agent and 0.1 per cent vanilla concentrate on a reconstituted basis gave satisfactory results. This cream-mix, when whipped by aeration, produced results similar to those obtained with the undried product.

There were no noticeable differences in whipping properties of reconstituted whipped cream-mix made from cream-mix sprayed with nozzle nos. 64, 72 and 79. The capacity of the drier was lowered by using nozzles with smaller orifices. The packing density was increased by using nozzles with larger orifices.

High spray pressures were found to be detrimental to the whipping properties of the reconstituted cream-mix, producing a product lacking in stability, containing large gas cells and having a moist appearance.

The keeping quality of the powdered cream-mix can be improved by packing in inert gas and by the addition of certain antioxidants before drying. As with powdered milk, it is desirable when storing the powdered cream-mix in inert gas to obtain a low oxygen content of the headspace on desorption. The antioxidants seemed to be more effective when the samples were packed in inert gas than in air. The most effective antioxidant was pure vanilla concentrate. Others that were effective were NDGA, gallic acid and Viobin.

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# EFFECT OF DELAY IN DILUTING AND COOLING ON KEEPING QUALITY OF BULL SEMEN

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Technique used in the handling of bull semen and in the breeding of cows artificially has made great progress in the last decade. Even so, many problems still confront workers in artificial breeding. Although improved diluters are now in use and the cooling of semen to between 35 and 40° F. is almost universally practiced, very little experimental work has been done on the time effect of immediate or delayed dilution and cooling. Probably the standard practice is to collect the semen and try to hold it as near the ejaculation temperature as possible until returning to the laboratory, where cooling and diluting procedures are inaugurated. The time required for collecting two ejaculates from a bull and returning to the laboratory, of course, varies according to how far the bulls are from the laboratory and how quickly the ejaculates may be collected from each individual bull. In some cases this time may be negligible, but in other cases enough time undoubtedly is consumed that it may mean the difference between semen that would rate "good" and "poor".

Reports on actual experiments testing the effects of delay in diluting or cooling of semen have not been found. However, procedures described by various workers (2, 3, 5, 6, 7, 8, 11) indicate that diluting and cooling should immediately follow collection and that cooling should be done slowly. One report (4) gives evidence that it is not necessary to cool the semen slowly.

In Louisiana and other southern states the temperature shock to spermatozoa, particularly that due to cold weather, probably is not as important as it is in more northern climates. This study was conducted in an effort to determine if the keeping qualities of bull semen are affected by the time between ejaculation and dilution and the beginning of cooling.

## EXPERIMENTAL PROCEDURE

Forty-two ejaculates from six different dairy bulls (five Holsteins and one Jersey) were used for this study. Following collection of each sample, three 1-ml. samples were taken from it for the experiment. These samples were treated as follows: No. 1, the diluting and cooling procedures were started immediately; no. 2, the semen was diluted immediately but the cool-

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ing procedure was not started until 45 minutes following collection; no. 3, both the diluting and the start of the cooling procedure were delayed for 45 minutes from time of collection.

The experiment was conducted between February 18 and April 29, 1947, inclusive. On the 11 actual experimental days during this period the air temperature as reported by the Baton Rouge weather bureau at 10:30 a.m. ranged from 44 to 80° F. and averaged 63.6° F.

The fresh semen was examined microscopically and rated into classes as described by Herman and Swanson (4), except that evaluations were made on the basis of 0.5 intervals. The same system of rating the samples was followed after storage at 35–40° F. for 24 and 72 hours. A variation of the methylene-blue test (1) was run on all samples, initially, and 24 and 72 hours after collection. One milliliter of fresh semen was mixed with 9 ml. of diluter. By using a small test tube, 1 ml. of methylene-blue solution was added to the diluted semen and mixed well. A 0.5-inch layer of mineral oil was added on top of the mixture to seal the tube, which then was placed in a water bath at 40° C. and the reduction time carefully noted. A variation of Beck and Salisbury's test (1), incubating the samples at 115° F. for 15 minutes and examining microscopically, was used on all samples 72 hours after collection.

The first 19 semen samples were diluted with synthetic pabulum (8) and the remaining 23 samples with yolk-citrate diluent (9). In no case were the two diluents used on portions of the same semen sample. All samples were diluted at the rate of 1:10, using 1 ml. of semen to 9 ml. of diluent, which first had been warmed to around 85 to 90° F.

A thermos bottle one-half full of water cooled to 35° F. was used when procedure called for the beginning of cooling immediately after collection. By slipping the vial of diluted semen partially through a hole in the thermos bottle stopper, gradual cooling was begun immediately. After returning to the laboratory, the temperature was taken and the vials of semen placed in 400-ml. beakers one-half full of water at a temperature the same as that of the semen. The beakers were placed in a refrigerator set at 35–40° F. and continued to be cooled until the desired storage temperature of about 38° F. was reached.

Where cooling was to be delayed 45 minutes—*e.g.*, until returning to the laboratory—the semen was diluted in a test tube and placed in 400-ml. beakers one-half full of water at about 85° F. The large beakers containing tubes of semen then were placed in a refrigerator and gradually cooled over a period of 2 to 2.5 hours to temperatures ranging from 35 and 40° F.

In analyzing the results of this study an analysis of variance (10) was run separately on: (a) Motility 24 hours after collection, (b) motility 72 hours after collection, (c) methylene-blue reduction time 24 hours

TABLE 1

*Comparison of motility ratings after storage for 24 and 72 hours between semen samples processed immediately and those delayed in diluting and cooling (Mean of 42 samples)*

| Procedures | Mean motility rating | | |
|---|---|---|---|
| | After storage for 24 hr. | After storage for 72 hr. | 72 hr. storage and incubation |
| No. 1, immediate diluting and cooling | 3.13 | 2.20 | 1.33 |
| No. 2, diluted immediately, cooling delayed 45 min. | 3.11 | 2.04 | 1.12 |
| No. 3, diluting and cooling delayed 45 min. | 2.96 | 1.81 | 1.02 |

after collection, (d) methylene-blue reduction time 72 hours after collection, and (e) incubation test 72 hours after collection.

## RESULTS

*Motility ratings.* Only slight differences in motility ratings (table 1) were noted between the averages for the three procedures based on observations made after 24 hours of storage. The mean value of 3.13 for no. 1 (immediate diluting and cooling) and 3.11 for no. 2 (immediate diluting and delayed cooling) samples indicated little advantage for immediate cooling. A greater effect was evidenced by the delay for 45 minutes in both diluting and cooling (no. 3), in which case the average was 2.96. Differences found after 24 hours of storage were highly significant statistically ($P < 0.001$), as were those found after 72 hours of storage.

Following 72 hours of storage, the average motility ratings (table 1) for the three procedures were 2.20, 2.04 and 1.81, respectively. These differences were greater than those found after 24 hours of storage and indicated that a delay in cooling or in both diluting and cooling tended to lower semen quality. This trend also is shown in the motility averages for

TABLE 2

*Comparison of methylene-blue reduction time of semen samples treated differently following collection (Mean of 42 samples)*

| Procedures | Mean reduction time | |
|---|---|---|
| | After storage for 24 hr. | After storage for 72 hr. |
| | *(min.)* | *(min.)* |
| No. 1, immediate diluting and cooling | 15.76 | 19.21 |
| No. 2, diluted immediately, cooling delayed 45 minutes | 16.91 | 20.14 |
| No. 3, diluting and cooling delayed 45 minutes | 17.50 | 22.88 |

samples stored for 72 hours and then subjected to incubation at 115° F. for 15 minutes prior to examination. These averaged 1.33, 1.12 and 1.02, respectively, for the three procedures.

*Methylene-blue reduction time.* As in motility ratings, the time required to reduce methylene blue (table 2) likewise indicated advantages for the immediate dilution and cooling of the semen samples. After storage for 24 hours the reduction time for the three procedures was 15.76, 16.91 and 17.50 minutes and after 72 hours of storage 19.21, 20.14 and 22.88 minutes, respectively. While the trend was the same for the tests made at the two periods of time, only those differences found for the 24-hour period proved to be statistically significant ($P < 0.001$).

## SUMMARY

Forty-two ejaculates from 6 different bulls were used in an experiment to determine if the amount of time required to collect semen and get it to the laboratory before dilution and beginning of cooling would affect the keeping qualities of the semen.

Using motility ratings from microscopic examinations made after 24 and 72 hours of storage before and after incubation and methylene-blue reduction tests as criteria of semen quality, it was found that the immediate processing of semen following collection was desirable for the maintenance of desirable characteristics of semen. Delay in either the diluting or the start of the cooling process tended to lower ratings made of the semen.

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# EFFECT OF FEEDING TOCOPHEROLS TO DAIRY COWS ON THE QUANTITY AND THE FAT CONTENT OF MILK PRODUCED[1]

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Preliminary results obtained in an experiment designed to determine the effect of feeding mixed tocopherols to dairy cows on their milk and butterfat production do not agree with those presented recently by Harris *et al.* (1). These workers reported that the feeding of mixed tocopherols at the rate of 1.0 g. daily per cow brought about an increase of about 27 per cent in the fat concentration and 21 per cent in the total quantity of milk (4 per cent fat-corrected) produced. Their experiment, however, was carried on in a privately owned herd and the milk was tested for butterfat content on only 2 days each month.

## EXPERIMENTAL

Seventeen purebred cows, ten Jerseys and seven Holsteins, of various ages were used in the present study. They were divided into two groups with eight cows in the control and nine in the supplemented group. The breed and age of cows used in both groups and their further division into classes A, B and C according to dates of calving are indicated in table 1.

The experiment started with a 10-day preliminary period, which ended May 21, 1947. During this period, none of the cows in either group was fed the tocopherol supplement. Feeding of the supplement, known as "Myvadry", to cows in the supplemented group was started on the evening of May 21 and continued through July 5, 1947. As in the experiment of Harris *et al.* (1), the supplement was added to the grain ration at time of feeding, in such amounts as to provide each cow with 1.0 g. of mixed tocopherols daily.

Cows in both groups were fed and managed alike except for the supplement. All cows were fed normal herd rations, which included good quality alfalfa hay and corn silage, along with concentrates in amounts according to their milk production. The Jerseys were fed grain at the rate of 1 lb. to every 2.5 to 3.5 lb. of milk produced and the Holsteins 1 lb. to every 4 to 5 lb. of milk produced daily. The grain mixture contained 225 lb. ground corn, 300 lb. ground oats, 300 lb. ground barley, 75 lb. linseed meal, 75 lb. soybean oil meal, 10 lb. steamed bone meal, and 15 lb. salt. When weather permitted, cows were kept outdoors except when being fed. Beginning on June 5, all the cows in both groups were turned on pasture

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daily but they continued to receive other roughage and the usual amounts of grain.

Records were kept of the amount and the fat content of milk produced daily by each cow during the entire period of the experiment, including the 7 days after feeding of the supplement had been discontinued. The Babcock method was used in determining the per cent of fat in the milk. Table 2 shows the average per cent of fat in the milk and the calculated average daily production per cow in pounds of 4 per cent fat-corrected milk of cows in each class and group during the preliminary period and at

TABLE 1

*Breed, date of birth and date of calving of cows used in the experiment*

| Animal no. | Breed | Class[a] | Date of birth | Date of calving |
|---|---|---|---|---|
| | | Control group | | |
| 256 | Jersey | A | 1–24–39 | 3–28–47 |
| 295 | " | A | 12–20–42 | 4– 6–47 |
| 302 | " | A | 2–27–44 | 3–28–47 |
| 275 | Jersey | B | 7– 2–40 | 12–26–46 |
| 299 | " | B | 11–10–43 | 12–23–46 |
| 474 | Holstein | C | 12–23–37 | 2–20–47 |
| 813 | " | C | 4– 8–41 | 1–13–47 |
| 838 | " | C | 11–17–43 | 1–15–47 |
| | | Supplemented group | | |
| 282 | Jersey | A | 9–20–41 | 4–12–47 |
| 300 | " | A | 12–18–43 | 4– 1–47 |
| 267 | Jersey | B | 12–15–39 | 1–31–47 |
| 280 | " | B | 6–26–41 | 1–23–47 |
| 297 | " | B | 2–24–43 | 1– 6–47 |
| 480 | Holstein | C | 3– 2–38 | 2– 3–47 |
| 492 | " | C | 11–21–39 | 1– 5–47 |
| 814 | " | C | 5–14–41 | 12–25–46 |
| 835 | " | C | 5–25–43 | 2–27–47 |

[a] The calving dates of cows in Classes A, B and C in the control group are approximately the same as of those in the corresponding classes in the supplemented group.

5-day intervals while supplement was fed and also during the 7-day period after feeding of supplement was discontinued.

The condition, general appearance and appetite of all animals remained good throughout the entire period of the experiment, with no class or group showing any superiority in any respect. The data in table 2 fail to indicate any tendency of a rise in the fat content of the milk from the cows in the supplemented group after feeding of the tocopherol was started on the evening of May 21; neither is there any evidence of a drop in the fat percentage during the 7 days of observation after feeding of the supplement was discontinued on July 6. Likewise, the supplementation had no

TABLE 2

*Average per cent of fat in milk produced and daily milk production (4% fat-corrected milk) of cows by classes and by entire groups during the indicated periods*[a]

| Group | Class | May | | | June | | | | | | July | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 12–21 | 22–26 | 27–31 | 1–5 | 6–10 | 11–15 | 16–20 | 21–25 | 26–30 | 1–5 | 6–12 |
| | | | | | *% of fat in milk* | | | | | | | |
| Control | A | 4.73 | 4.73 | 4.52 | 4.60 | 4.97 | 5.09 | 4.75 | 4.80 | 4.69 | 4.60 | 4.72 |
| Supplemented | A | 4.64 | 4.37 | 4.49 | 4.37 | 4.74 | 5.00 | 4.61 | 4.61 | 4.50 | 4.64 | 4.48 |
| Control | B | 5.13 | 5.21 | 5.32 | 5.23 | 5.63 | 5.58 | 5.58 | 5.49 | 5.18 | 5.38 | 5.37 |
| Supplemented | B | 4.88 | 4.64 | 4.82 | 4.81 | 5.18 | 5.25 | 5.07 | 4.94 | 4.86 | 4.96 | 4.99 |
| Control | C | 3.12 | 3.07 | 3.12 | 3.12 | 3.13 | 3.23 | 3.04 | 2.96 | 2.95 | 3.09 | 3.04 |
| Supplemented | C | 3.15 | 3.22 | 3.18 | 3.24 | 3.20 | 3.26 | 3.21 | 3.10 | 2.92 | 3.17 | 2.95 |
| Control | All | 4.14 | 4.10 | 4.07 | 4.09 | 4.27 | 4.37 | 4.13 | 4.11 | 4.00 | 4.09 | 4.10 |
| Supplemented | All | 3.87 | 3.86 | 3.93 | 3.90 | 4.10 | 4.18 | 4.03 | 3.93 | 3.79 | 3.98 | 3.84 |
| | | | | | *lb. 4% fat-corrected milk* | | | | | | | |
| Control | A | 31.8 | 29.8 | 28.0 | 26.9 | 30.8 | 30.3 | 28.7 | 28.7 | 26.2 | 24.4 | 25.1 |
| Supplemented | A | 37.6 | 36.8 | 35.9 | 35.0 | 38.6 | 38.7 | 36.5 | 34.1 | 30.5 | 28.4 | 28.9 |
| Control | B | 21.4 | 19.8 | 20.0 | 19.7 | 22.1 | 22.5 | 21.2 | 21.4 | 18.3 | 19.9 | 18.2 |
| Supplemented | B | 21.7 | 21.4 | 20.0 | 20.0 | 23.4 | 23.3 | 22.6 | 22.4 | 20.4 | 20.2 | 20.4 |
| Control | C | 25.0 | 24.5 | 23.5 | 23.5 | 26.4 | 26.6 | 25.6 | 24.7 | 22.8 | 22.2 | 21.9 |
| Supplemented | C | 28.6 | 26.3 | 24.9 | 26.5 | 26.9 | 28.5 | 26.6 | 26.7 | 23.9 | 23.3 | 22.4 |
| Control | All | 26.6 | 25.3 | 24.3 | 23.8 | 27.0 | 27.0 | 25.7 | 25.4 | 22.9 | 22.6 | 22.3 |
| Supplemented | All | 28.2 | 26.3 | 25.7 | 26.2 | 28.4 | 29.0 | 27.5 | 26.9 | 24.2 | 23.6 | 23.3 |

[a] None of the cows in either group was fed the tocopherol supplement during the first and the last periods indicated.

apparent effect on the quantity of milk produced, as expressed in terms of 4 per cent fat-corrected milk. Although the cows in the supplemented group produced at a somewhat higher level than those in the control group, this difference was as great during the preliminary period as it was later during the time when the supplement was fed.

Both the per cent of fat in the milk and the amounts of 4 per cent fat-corrected milk produced by cows in each class and group varied widely from period to period during the progress of the experiment. These variations, however, are no more marked in one class or group than in the other; furthermore, they show a definite tendency to occur simultaneously in the several classes and groups as though resulting from a common cause. Such an effect is indicated by the marked increase in milk production and to a less marked degree by the rise in the per cent of fat in the milk produced by cows in all classes of both groups after they were turned on pasture on June 5.

A more comprehensive study of this problem is under way, the results of which will be presented at some later date.

## SUMMARY AND CONCLUSIONS

A feeding trial was conducted to determine the effect of supplementing the rations of lactating dairy cows with 1 g. of mixed tocopherols daily per cow on the amount and fat content of the milk produced. Seventeen cows were used, eight of which received basal ration and the remainder received supplemental tocopherol daily from May 21 to July 5, inclusive. Milk from each cow in both groups was weighed and tested for butterfat content daily.

Neither the amount nor the fat content of the milk produced appeared to be affected by feeding of the tocopherol supplement. Such supplementation produced no changes in the appearances and appetites of the animals.

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# THE ELIMINATION OF INTERFERING SUBSTANCES IN THE KAY–GRAHAM PHOSPHATASE TEST WHEN USED FOR HARD RIPENED CHEESE[1]

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The Kay-Graham phosphatase test (5), including the Gilcreas and Davis modification (4), is not suitable for the analysis of hard ripened cheese. A recent report by Gilcreas (3) comparing the effectiveness of several phosphatase tests in the examination of hard cheese emphasized this point when he stated the following concerning the Kay-Graham method: ". . . the control values particularly in the examination of aged cheese are so high as to limit sharply the utility of this test for detecting the presence of the active enzymes in the sample. This interference is undoubtedly caused by amino acids, particularly tyrosine which is always present in aged cheese." Other phosphatase tests such as those of Sanders and Sager (8) and Scharer (9) would not encounter this type of interference as 2,6-dibromo-quinone-chloroimide (BQC), the color reagent used for these tests, is specific for phenol, whereas the Folin-Ciocalteu color reagent used in the Kay-Graham test originally was developed to determine tyrosine and tryptophane (2).

Results from recent investigations upon the protein decomposition products of cheese (1, 6) encouraged the authors to believe that all the interfering substances could be eliminated in the application of the Kay-Graham test to cheese. It was obvious that tyrosine and possibly tryptophane are interfering substances and that the amine, tyramine, which was shown (6) to be present in all cured commercial Cheddar cheese, also contributed greatly to the interference. This amine is water soluble and forms a blue compound with the Folin-Ciocalteu reagent. In the standard Kay-Graham method it passes into the filtrate to intensify the color.

To eliminate all interfering amino acids and amines from the Kay-Graham test, certain solubility principles were considered. Tyrosine and tryptophane, as well as most of the other amino acids, are insoluble in ether. Tyramine is soluble in ether when extracted under slightly alkaline conditions, but it is insoluble in ether under acid conditions. Phenol, on the other hand, is very soluble in ether under acid conditions.

## EXPERIMENTAL RESULTS

Utilizing the solubility characteristics of the various compounds in-

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volved, the following approach, designated as the trichloracetic acid technic, was evolved.

*The method.* A sample of ground cheese (0.5 g.) was incubated with 10 ml. of buffer substrate and two drops of chloroform for 24 hours at 37° C. The buffer substrate contained 1.09 g. disodium phenyl phosphate and 17.54 g. of sodium barbital per liter of water, to which 10 ml. of chloroform were added. Following this incubation, 1 ml. of a 25 per cent trichloracetic acid solution was added. The resulting precipitate was filtered off through Whatman no. 42 filter paper. Five milliliters of the clear filtrate then were pipetted into a standard Mojonnier extraction flask. Enough 1 per cent hydrochloric acid solution was added to this flask to bring the liquid to the bottom of the neck of the flask. Then 25 ml. of ethyl ether were added. The Mojonnier flask was stoppered with a cork covered with tinfoil and inverted slowly 20 times. After the agitation was completed, the clear ether was poured off into a large tube containing 5 ml. of distilled water. The ether was boiled off in about 5 minutes by immersing the tube in a beaker of hot water.

To the 5 ml. of remaining aqueous solution, 2 ml. of Folin-Ciocalteu reagent (diluted 1 to 2) were added, followed immediately by 2 ml. of 14 per cent sodium carbonate. The mixture was placed in boiling water for 5 minutes, after which it was cooled, filtered and the color readings taken. A Luximeter colorimeter was used throughout the entire experimental work.

*Phosphatase and interfering blank values of Cheddar cheese.* Data were obtained on an assorted group of 25 representative commercial Cheddar cheeses. In table 1, columns 4 and 5, the phosphatase values and interfering blank values of the different cheeses are shown as obtained by the trichloracetic acid technic. The interfering blanks were obtained by using a plain buffer solution without the disodium phenyl phosphate substrate and by eliminating the incubation period which showed the possibility of removing interfering substances present in the original cheese. Otherwise the method was similar. Controls also were run on many of the cheeses. In column 6, table 1, are shown the interfering blank values obtained on the cheeses by the standard method of Kay-Graham (4, 5) using a 0.5-g. sample of cheese.

The phosphatase values obtained by the trichloracetic acid technic show that a wide variation existed among these cheeses. Some of these tested cheeses were made from raw milk and some were made from pasteurized milk. The important fact is that all the cheeses tested, ranging in age from 7 months to 10 years, showed interfering blank values which were extremely low, varying from 0.002 to 0.009 mg. phenol per 0.5 g. of cheese and averaging 0.004 mg. per 0.5 g. of cheese. Control values on the cheeses tested also were extremely low. The final filtrates in the trichloracetic

technic always were clear blue, being very similar in character to those obtained on milk. It was very easy to read intensity differences.

The interfering blank values obtained using the standard Kay-Graham method (table 1), with one exception, were all extremely high, indicating gross contamination by interfering substances produced during ripening.

TABLE 1

*The phosphatase and interfering blank values of 25 commercial Cheddar cheeses obtained by the trichloracetic acid modification of the Kay-Graham method, the Standard Kay-Graham method, and the Sanders-Sager method*

| Cheese | | | Trichloracetic acid technic | | Standard Kay-Graham method | Sanders-Sager method[a] |
|---|---|---|---|---|---|---|
| No. | Age | Mfgs. report | Phosphatase values | Interfering blank values[b] | Interfering blank values[b] | Phosphatase values |
| | (*mo.*) | | (*mg. phenol/0.5 g. cheese*) | | (*mg. phenol/0.5 g. cheese*) | (*γ phenol/0.25 g. cheese*) |
| 1 | 8 | Past. | 0.013 | 0.004 | 0.144 | 2.5 |
| 2 | 19 | Past. | 0.010 | 0.006 | 0.265 | 3.0 |
| 3 | 8 | Past. | 0.014 | 0.003 | 0.188 | 3.0 |
| 4 | 17 | Past. | 0.012 | 0.005 | 0.302 | 1.0 |
| 5 | 18 | Past. | 0.013 | 0.002 | 0.271 | 2.0 |
| 6 | 10 | Past. | 0.004 | 0.002 | 0.360 | 2.0 |
| 7 | 8 | Past. | 0.020 | 0.002 | 0.225 | 5.0 |
| 8 | 11.5 | Past. | 0.046 | 0.004 | 0.200 | 6.0 |
| 9 | 8 | Past. | 0.045 | 0.005 | 0.122 | 6.0 |
| 10 | 13 | Past. | 0.060 | 0.004 | 0.350 | 9.0 |
| 11 | 14 | Raw | 0.698 | 0.003 | 0.331 | 35.0 |
| 12 | 126 | Raw | 0.785 | 0.007 | 0.703 | > 40.0 |
| 13 | 8.5 | Raw | 1.226 | 0.006 | 0.241 | > 40.0 |
| 14 | 16 | Raw | 0.821 | 0.004 | 0.382 | > 40.0 |
| 15 | 15 | Raw | 0.769 | 0.006 | 0.422 | 40.0 |
| 16 | 38 | Raw | 0.745 | 0.006 | 0.466 | 30.0 |
| 17 | 7 | Raw | 0.849 | 0.004 | 0.181 | > 40.0 |
| 18 | 41 | Raw | 0.702 | 0.003 | 0.703 | 40.0 |
| 19 | 35.5 | Raw | 0.978 | 0.006 | 0.497 | > 40.0 |
| 20 | 13.5 | Raw | 0.773 | 0.006 | 0.125 | > 40.0 |
| 21 | 8 | Raw | 0.853 | 0.002 | 0.038 | > 40.0 |
| 22 | 7 | Raw | 0.637 | 0.003 | 0.166 | > 40.0 |
| 23 | 8 | Raw | 0.853 | 0.009 | 0.196 | > 40.0 |
| 24 | 29 | Raw | 0.749 | 0.009 | 0.396 | 35.0 |
| 25 | 16 | Raw | 0.702 | 0.004 | 0.125 | > 40.0 |

[a] A value of over 3.0 for the Sanders-Sager method indicates cheese made from improperly pasteurized milk.

[b] Shows amount of interfering substances developed in cheese during ripening and not removed by the trichloracetic acid technic.

The range extended from 0.038 to 0.703 mg. phenol per 0.5 g. cheese. In cheese of low phosphatase values, the interfering blank values often were as much as 60 times as great as the actual phosphatase value of the cheese.

Although it was evident that the interfering substances could be eliminated completely, there was as yet no evidence to show that by using this

technic it would be possible to follow differences in the phosphatase concentration of various cheeses comparable to those shown by standard phosphatase methods. To check this point, all the cheeses were tested by the phosphatase method of Sanders and Sager (8) and these results were compared to results using the new technic, even though there is now no intent of presenting a final procedure for the new technic. The dilution procedure for the Sanders-Sager method which would make the results of those cheeses containing more than 40 $\gamma$ of phenol per 0.25 g. cheese more quantitative was omitted although this omission does not prevent a correct interpretation of these results. The data, presented in table 1, include the heat treatment history of the cheese milk and the age of the various cheeses, both as given by the manufacturers. It was evident from the results obtained by the Sanders-Sager phosphatase test that some of the cheeses, stated to have been made from pasteurized milk, actually were made from underpasteurized milk. Although at the present time data are not available to show at what concentration of phenol the trichloracetic acid technic distinguishes raw from pasteurized milk cheese, it is encouraging to note that where there are phenol-value increases in the Sanders-Sager method (table 1), there also are phenol-value increases in the trichloracetic acid modification (column 4, table 1) of the Kay-Graham method, although not necessarily of the same magnitude. With a few exceptions, the phenol values of the cheese were related to the reported pasteurization of the milk.

The magnitude of the phenol values for cheese obtained by the trichloracetic acid technic corresponds relatively closely to that obtained on milk with the standard Kay-Graham method, although the critical value dividing the raw from the pasteurized product does not appear to be quite the same.

*Interfering substances other than tyrosine and tyramine.* It has been reported by Leahy *et al.* (7) that the Folin-Ciocalteu reagent also gave strong colors with a variety of chemical compounds including diacetyl, acetylmethylcarbinol, cystine, l-leucine, indole, uric acid, allantoin and quanine. Although none of these compounds has been reported in cheese in significant amounts, it may be well to point out that most of them are insoluble in ether and others are insoluble in aqueous solutions. However, diacetyl is considered to be soluble in both ether and water. Although only traces have ever been reported in cheese, a study was made to note the effect of the addition of small amounts to cheese. The amounts selected were one to ten times that usually found in ripened butter, which contains on the average of about 3–4 p.p.m. No significant increase was noted in the final readings after these additions, indicating even if diacetyl were ever present it would not affect the final results. The very fact that it was possible to test 25 cheeses of vastly different history and obtain low blanks is another indication that these interfering substances are no longer significant using the modified method.

## DISCUSSION

The Kay-Graham phosphatase test as commonly used for milk has been found inaccurate on ripened hard cheeses because of the non-specificity of the Folin-Ciocalteu reagent toward phenol. As the Kay-Graham test is so valuable in the dairy industry, any attempt to overcome its inaccuracy on cheese should be encouraged. Although at this time no attempt has been made to present a routine method for the Kay-Graham phosphatase test on cheese, the removal of the interfering substances should stimulate development of such a method. Additional information is being gathered to perfect the details of the new technic and to show the adaptability and sensitivity of this technic in distinguishing cheeses made from milk heated at different times and temperatures.

The principle employed for the elimination of interfering substances actually consisted of producing conditions which allowed for the selective removal of most of the free phenol produced. The free phenol was produced first in an alkaline medium by the phosphatase enzymes and then extracted with ether under acid conditions so that none of the amino acids or amines likely to produce interference would be extracted. Removal of free phenol by washing with ether is a common practice in the purification of some chemical reagents and biological materials containing phenolic substances. It then was possible to retain the phenol in an aqueous solution by boiling off the ether. Because of its high boiling point (182° C.), probably very little phenol would be lost.

There is no doubt that tyramine is a very important factor contributing to the blue interference, as every cheese in this series contained it in some concentration. If any other products derived from the amino acids or other chemical substances were responsible for interference, they also are ether insoluble, as almost negative interfering blanks and controls were obtained using the trichloracetic technic.

The use of trichloracetic acid was found very desirable because it possesses flocculating properties without requiring the aid of heat. It also aided in better color development for some unknown reason. In addition, a clear filtrate always was obtained prior to extraction. However, trichloracetic acid is soluble in ether and in order to keep the filtrate at pH 1–2 during the extraction, an inorganic acid was added.

Occasionally an emulsion was encountered in the extraction process. This easily was broken by running hot tap water over the lower chamber of the Mojonnier flask for about 20 seconds with the cork loosely stoppered, followed by rapid cooling under the cold water tap.

The standard Kay-Graham method for milk expresses the results in units of *mg. phenol per 0.5 ml. milk* and uses only an aliquot portion of 0.5-ml. milk in the test. In the interests of uniformity the unit used for the trichloracetic acid modification is *mg. phenol/0.5 g. cheese* even though

here again an aliquot sample smaller than 0.5 g. cheese is used for the final color determinations. For basic quantitative measurement of phenol in certain studies all dilutions should be considered. This can be done easily in this modification, if desired, but in the case of the phosphatase test where the method is mainly empirical in nature this is not considered essential.

## SUMMARY

A technic was developed for the selective separation of free phenol from interfering substances in the Kay-Graham phosphatase test when used on hard ripened cheese. Tyrosine and tyramine were important interfering substances.

The elimination of the interfering substances was accomplished by using trichloracetic acid as a precipitant for the cheese proteins, and by extracting the free phenol, formed as a result of phosphatase activity in an alkaline substrate, with ether under acid conditions using a Mojonnier-type extractor. The ether containing the phenol was placed in distilled water and then was boiled off. The aqueous solution then was treated with the Folin-Ciocalteu reagent and the amount of phenol determined with a colorimeter.

The amount of interfering substances in ripened cheese not removed by using the new trichloracetic acid technic was extremely low, as values averaging 0.004 mg. phenol per 0.5 g. cheese were obtained for 25 cheeses varying in age from 7 months to 10 years.

Phenol values obtained on cheese using the new technic showed changes in phosphatase activity which corresponded well with results obtained on the same cheeses with the Sanders-Sager phosphatase method. The phenol values obtained using this new technic on cheese were on the order of those obtained on milk using the standard Kay-Graham method, but no complete data have been obtained yet to establish the final details of the test and to show at what point the new technic would distinguish raw from pasteurized milk cheese.

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# DEHYDRATED SWEET POTATOES AS A CONCENTRATE FEED FOR DAIRY CATTLE[1]

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Selection of the best quality sweet potatoes for table use yields culls which may be dehydrated and used for animal feed. High yields of sweet potatoes in some areas may justify growing them specifically for animal feeding, especially where corn yields are low. The present study was undertaken to determine the value of dehydrated sweet potatoes as a concentrate feed for dairy cattle.

## REVIEW OF LITERATURE

Feeding trials conducted with fattening pigs (9, 10, 18) indicated that dehydrated sweet potatoes did not produce satisfactory gains, largely due to low palatability and a laxative effect.

For fattening steers, more satisfactory results have been obtained. When sweet potatoes replaced all of the corn in the ration, gains were less rapid than on corn (10, 11, 17) and were less efficiently made (10, 11). In a mixed ration, sweet potatoes were found equal to corn except for the lower protein content (7). In comparison with corn and wheat, gains were as rapid on sweet potato rations but the appraised value was slightly lower (6). When replacing only 50 per cent of the corn, sweet potatoes gave more rapid gains and the selling price on the steers was higher (17).

For dairy cows, Copeland (5) found that dried sweet potatoes were 90.75 per cent as valuable as corn for milk production but that the butter from sweet potato-fed cows had 37.98 I.U. per g. of vitamin A whereas that from the corn-fed cows contained only 31.11 I.U. per g. Trials with dairy cows in Louisiana (14, 16) showed that dehydrated sweet potatoes have approximately 88 per cent of the value of yellow corn meal, but are approximately 17 per cent more valuable than ground snapped corn, including cob and shuck.

Digestion trials on dehydrated sweet potatoes have been carried out with steers and lambs (3) and with dairy cows (14, 16). Briggs *et al.* (3) found that with steers the digestibility of the nitrogen-free extract of sweet potatoes was 93.4 per cent when fed with prairie hay and cottonseed meal and 98.5 per cent when fed with alfalfa hay. With lambs the correspond-

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ing values were 87.8 and 92.4 per cent. On a dry matter basis, the total digestible nutrients ranged from 78.7 to 91.4 per cent.

Rusoff *et al.* (14) and Seath *et al.* (16) had difficulty in obtaining the apparent digestibility of fat, fiber, and protein in dehydrated sweet potatoes, but in four trials found digestibility coefficients of 83.47 to 94.46 per cent for the nitrogen-free extract, and calculated total digestible nutrient values from 71.78 to 81.06 per cent on the dry basis. The lower values were for off-grade sweet potatoes which had been cut and bruised and heated to a higher temperature than commonly used.

## EXPERIMENTAL PROCEDURE

In this experiment two methods were used to determine the feeding value of dehydrated sweet potatoes. First, a 75-day trial was conducted to determine the milk-producing properties relative to ground yellow corn. Secondly, a digestion trial was conducted to determine digestibility of the main components in order to estimate the total digestible nutrients for more direct comparison with other carbohydrate feeds.

*Milk production trial.* Four groups of three Holstein cows each were used in the milk production trial. The cows had been fresh from 41 to 142 days at the beginning of the trial; the cow which had been fresh for only 41 days had reached her peak production. These were grouped according to age (six mature cows and six first-calf heifers) and according to milk production within the age groups. Three rations were fed as indicated below. After an equalization and standardization period of 2 weeks, the cows were placed on the experiment, which consisted of three periods of 25 days each, of which the first 5 days was a transition period and the remaining 20 days the experimental period.

The animals were fed alfalfa hay and corn silage at a rate of approximately 8 lb. of hay and 24 lb. of silage per 1,000 lb. of body weight. Grain was fed at first at rates based on the grain feeding table (Appendix Table IXa) in *Feeds and Feeding* (13) for cows receiving the 1.5-lb. hay equivalent, but was adjusted so as to maintain production at approximately the level previous to adjustment. More grain was allowed those animals in their first lactation to allow for growth requirements. Concentrate allowances ranged from 11.6 to 21.0 lb. per day.

Following the suggestion of Lucas (12), the amount of concentrates for all cows was reduced at a rate uniform for all animals regardless of the ration received. As the decrease in production was very slight after the first 2 weeks, no adjustment was made until the end of the first feeding period, at which time the concentrate allowance was reduced 1.5 per cent. The same reduction was made at the end of the second period.

Three rations were used. Ration *A* consisted of ground shelled corn and soybean meal in a ratio of 650 lb. ground corn to 125 lb. soybean meal.

Ration *B* consisted of 325 lb. ground corn, 325 lb. dehydrated sweet potatoes and 125 lb. soybean meal. Ration *C* consisted of 650 lb. dehydrated sweet potatoes plus 125 lb. soybean meal. This proportion of soybean meal was calculated as supplying sufficient protein to the cows on ration *C*, and some excess to those on rations *A* and *B*. Because of some difficulty in grinding the dehydrated sweet potatoes with the high moisture content when received, the sweet potatoes were fed as they came from the bag, in shreds about 0.25-inch square by 1 to 2 inches long. For cows on ration *B* the sweet potatoes were weighed separately, and the corn and soybean meal mixed in the proper proportion were weighed separately. For cows on ration *C*, the sweet potatoes and soybean meal were weighed separately.

The sweet potatoes used in dehydration were principally the Maryland Golden variety, but small amounts of Jersey-type sweet potatoes were mixed with them. They consisted of the culls, such as the jumbos, small sized, cut and bruised sweet potatoes. All were cleaned and free of decay.

The ration sequences were arranged according to the method of Cochran

TABLE 1

*Organization of milk production trial showing ration sequences*

| Period | Groups I and III | | | Groups II and IV | | |
|---|---|---|---|---|---|---|
| | Cow 1 | Cow 2 | Cow 3 | Cow 1 | Cow 2 | Cow 3 |
| I | A | B | C | A | B | C |
| II | B | C | A | C | A | B |
| III | C | A | B | B | C | A |

*et al.* (4) to allow for measurement of carry-over effects if these should persist beyond the 5-day transition period. The sequences were arranged as shown in table 1.

Within each group, the particular sequences were assigned to the cows at random.

Milk weights were recorded for each milking. A 1-day composite was accurately prepared once in each 5-day subperiod for a Babcock test. On the last day of each main period a carefully composited sample of milk was prepared for butterfat test, carotene and vitamin A analysis. For the carotene and vitamin A analysis, the method of Boyer *et al.* (2) was used with slight modification. After the extract was concentrated, it was passed through a small chromatograph of sodium carbonate (Frank W. Kerr Co., Detroit, Mich.). Carotene and vitamin A then were determined as recommended. At the same time, blood samples were drawn and the plasma analyzed for carotene and vitamin A according to the method of Boyer *et al.* (1), also with slight modifications. Only 5 ml. of plasma were extracted. After precipitation of the carotene-fat mixture, the samples were stored in the refrigerator until the precipitate had clumped, to facilitate filtration. This storage has not been found to reduce the vitamin A.

Body weights were determined at the end of each period by weighing on three successive days.

*Digestion trial.* Four mature Holstein cows nearing the end of their lactation periods were dried off 3 to 4 months before they were due to calve. Their requirements for maintenance were estimated according to the Morrison standard, with no special allowance for gestation. The amounts of mixed timothy-clover hay necessary to meet these requirements then were calculated. Two of the cows were placed on the hay ration exclusively. The other two cows received one-half of the calculated amount of hay and an equal number of pounds of dehydrated sweet potatoes.

After 7 days on these rations, the cows were placed in the digestion stall room for the digestion trial carried out as described by Eheart *et al.* (8).

TABLE 2

*Analysis of variance of fat-corrected milk, average butterfat test, and body weight per period per cow*

| Source | Fat-corrected milk | | Butterfat test | | Body weight | |
|---|---|---|---|---|---|---|
| | Degrees freedom | Mean square | Degrees freedom | Mean square | Degrees freedom | Mean square |
| Period | 2 | 3,395 | 2 | 0.120* | 2 | 2,249** |
| Group | 3 | 122,416** | 3 | 0.113* | 3 | 47,101** |
| Period × group | 6 | 4,202* | 6 | 0.070 | 6 | 821* |
| Cows within groups | 8 | 9,905* | 8 | 0.196** | 8 | 26,627** |
| Ration | ..... | ..... | 2 | 0.045 | 2 | 1,599* |
| Direct (adjusted) | 2 | 14,180** | ..... | ..... | ..... | ..... |
| Residual (adjusted) | 2 | 774 | ..... | ..... | ..... | ..... |
| Error | 12 | 1,248 | 14 | 0.029 | 14 | 261 |
| Standard error per cow | ..... | 35.3 lb. | ..... | 0.170% | ..... | 16.2 lb. |
| Coefficient of variation (%) | ..... | 4.78 | ..... | 5.00 | ..... | 1.44 |

* Represents significance at the 5% point.
** Represents significance at the 1% point.

At the end of this period they were removed from the digestion stalls and changed to the other ration; those which had received hay only were cut to one-half the amount of hay and given an equal amount of sweet potatoes. Those which had been receiving hay and sweet potatoes were changed to hay only, in an amount equaling the total feed received previously. After 9 days the cows were returned to the digestion stalls for a second digestion trial. The changes in weight of the animals themselves were small and not significant.

## RESULTS

*Milk production trial.* The results of the analysis of variance of the data obtained on 4 per cent fat-corrected milk, butterfat test, body weight, blood plasma carotene, blood plasma vitamin A, milk carotene and milk vitamin A are presented in tables 2 and 3. This analysis follows that of

TABLE 3

*Analysis of variance of blood plasma carotene, blood plasma vitamin A, milk carotene, and milk vitamin A (γ/100 ml.)*

| Source | Degrees of freedom | Mean square | | | |
|---|---|---|---|---|---|
| | | Plasma carotene | Plasma vitamin A | Milk carotene | Milk vitamin A |
| Period | 2 | 16,088** | 80.44** | 62.92** | 793.29** |
| Group | 3 | 5,919* | 69.47** | 24.03 | 17.78 |
| Period × group | 6 | 1,940 | 4.32 | 43.26* | 9.75 |
| Cows within groups | 8 | 8,716** | 39.59** | 17.15 | 18.00 |
| Ration | | | | | |
| Direct (adjusted) | 2 | 33,806** | 138.12** | 111.52** | 60.06* |
| Residual (adjusted) | 2 | 507 | 10.60 | 2.86 | 0.38 |
| Error | 12 | 1,483 | 7.43 | 9.96 | 10.28 |
| Standard error per cow | ..... | 38.5 | 2.73 | 3.16 | 3.21 |
| Coefficient of variation (%) | ..... | 15.9 | 10.7 | 19.0 | 19.3 |

* Represents significance at the 5% point.
** Represents significance at the 1% point.

Cochran *et al.* (4) with the analysis for direct and carry-over or residual effects of the rations except in the case of butterfat test and body weight. These latter items were analyzed according to the usual procedure without breakdown into direct and residual effects. In no case did the residual effects even approach significance. Direct ration effects were significant at the 1 per cent point for fat-corrected milk, blood plasma carotene, blood plasma vitamin A and milk carotene. Direct ration effects were significant at the 5 per cent point for milk vitamin A. Ration effects were significant at the 5 per cent point for body weight but were not significant for the butterfat test.

Because the residual effects were slight and not statistically significant, the mean values actually obtained, without adjustment, are presented. The mean values for the three rations are presented in table 4 with percentage relationships based on ration *A* as 100.

TABLE 4

*Mean values obtained for rations A, B, and C, and percentage relationships based on ration A as 100*

| | Mean values | | | Percentage of ration A | |
|---|---|---|---|---|---|
| | Ration A | Ration B | Ration C | Ration B | Ration C |
| 4% fat-corrected milk[a] | 38.4 | 37.4 | 35.1 | 97.4 | 91.4 |
| Butterfat test (%) | 3.44 | 3.42 | 3.34 | 99.4 | 97.1 |
| Body weight (lb.) | 1115.3 | 1118.5 | 1136.7 | 100.3 | 101.9 |
| Plasma carotene (γ/100 ml.) | 170.3 | 277.2 | 279.8 | 162.8 | 164.3 |
| Plasma vitamin A (γ/100 ml.) | 21.8 | 26.1 | 28.5 | 119.7 | 130.7 |
| Milk carotene (γ/100 ml.) | 13.0 | 16.9 | 20.0 | 130.0 | 153.8 |
| Milk vitamin A (γ/100 ml.) | 13.9 | 17.0 | 19.0 | 122.3 | 136.7 |

[a] Lb. per cow per day.

*Digestion trial.* The digestion coefficients and their standard errors and also the average of seven analyses of the components of the dehydrated sweet potatoes used in the trial are given in table 5.

On the basis of the analysis of dehydrated sweet potatoes actually used in the digestion trials, and considering negative digestion coefficients as zero, the total digestible nutrients are 79.0 per cent on a dry matter basis or 69.6 per cent on a 12 per cent-moisture basis. Using the average of the seven analyses as presented in table 5, the total digestible nutrients are 80.0 per cent on the dry matter basis or 70.4 per cent on a 12 per cent-moisture basis.

TABLE 5

*Digestion coefficients and average chemical composition (7 analyses) of dehydrated sweet potatoes*

| Material determined | Digestion coefficients | Average composition (dry basis) |
|---|---|---|
| | *(%)* | *(%)* |
| Crude protein | 3.19 ± 3.11 | 4.86 ± 0.14 |
| Ether extract | 52.04 ± 5.74 | 0.82 ± 0.13 |
| Crude fiber | − 51.56 ± 21.61 | 3.25 ± 0.22 |
| Nitrogen-free extract | 90.08 ± 0.43 | 87.56 ± 0.47 |
| Ash | ..... | 3.49 ± 0.03 |

## DISCUSSION

The results of this experiment are in line with the results of other work with dairy cows, namely that of Copeland (5) and Rusoff *et al.* (14). In the present trial, when sweet potatoes replaced all of the corn, they were found to be 91.4 per cent as valuable, and when they replaced only half of the corn, they were found to be 94.8 per cent as valuable. On the basis of the calculated total digestible nutrient values, the sweet potatoes were 93.2 per cent as high as corn when compared with the values listed by Schneider (15) for corn grain, found by difference when using cattle for test purposes.

The results on carotene and vitamin A were as expected and corroborate the results of Copeland (5). Dehydrated sweet potatoes have special value for maintaining the carotene and vitamin A in the body and in the milk during the barn feeding period when the quality of the hay is poor, especially when no silage is available.

The standard error per cow and the coefficients of variation for the fat-corrected milk, butterfat test, and body weight were about normal for this type of experiment. In the case of blood plasma carotene and vitamin A, and milk carotene and vitamin A, the coefficients of variation were quite high, but the differences were large enough that the large error terms did not prevent obtaining statistically significant differences.

In the digestion trial the digestibility of the most important constituent, nitrogen-free extract, was high and the results with the four animals were quite uniform. The digestibility of protein was low and not significantly different from zero, but may be quite inaccurate due to the small amount present. The digestibility of crude fiber as determined with each of the four animals was a negative value, but the average was not significantly different from zero. Results on crude fiber at Oklahoma (3) and Louisiana (14) also were negative in some cases.

These results would seem to support the theory that with the increase in the amount of readily soluble carbohydrate in the rumen, the digestibility of the fiber actually may be decreased, through selective use by the rumen organisms. With the small amounts of protein, fat, and fiber present in the sweet potatoes, it might be said that the low digestibility is not important. However, since both the protein and fat already are low compared with corn, the low digestibility merely aggravates a shortage which must be made up by supplying other feeds.

The palatability of the sweet potatoes was quite satisfactory. Three of the cows ate up to 17.6 lb. of sweet potatoes per day in addition to 3.4 lb. of soybean meal. For short periods of time, one of these high-producing cows showed a tendency to leave some of the ration.

The only abnormal results of the experiment were obtained with one of the high-producing cows, whose feces became quite watery. Drug treatment failed to clear up the condition. The appetite was normal and the amount of milk produced continued the same. However, during the period when she was receiving the sweet potatoes, the butterfat test dropped well below normal, averaging 2.4 per cent for four tests. This one cow was largely responsible for the lower fat test for the sweet potato ration. After the discontinuation of the experiment, however, this cow remained in a diarrheic condition for quite some time; this might have been expected as she was turned to pasture after the experiment. In the digestion trial the feces did indicate a slightly more laxative effect for the sweet potato ration than the all-hay ration.

In some areas dehydrated sweet potatoes would seem to show definite possibilities in replacing part of the corn or other carbohydrate feed, if the lower protein and fat are taken into consideration in balancing the rations. At certain times of the year and for certain milk producers, the high carotene content has a special value above the value as a carbohydrate feed.

## SUMMARY

1. Dehydrated sweet potatoes when fed to dairy cows in a 75-day double change-over experiment were found to have 91.4 per cent the value of ground yellow corn when they replaced all of the corn. When they replaced only half of the corn, they were 94.8 per cent as valuable.

2. In a digestion trial using four mature dairy cows, the main constit-

uent, nitrogen-free extract, was found to have a digestibility of 90.08 ± 0.43 per cent. On the basis of the digestion coefficients found in this experiment, the total digestible nutrient value was found to be 70.4 per cent on a 12 per cent-moisture basis.

3. Dehydrated sweet potatoes were found to excel corn in maintaining a high level of carotene and vitamin A in the blood plasma and milk. Therefore, they sometimes would have special values which would counteract their slightly lower milk-producing value.

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# A SIMPLE COLOR TEST AS AN AID IN GRADING FARM-SEPARATED CREAM[1, 2]

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It is widely recognized by the creamery industry that a simple test to support the organoleptic method of grading cream would be highly desirable. Although the flavor and odor method is the most satisfactory means available for grading cream for butter making, it has several disadvantages when used by cream station operators as a basis of payment to producers. The chief criticisms are the variability in results due to the personal factor and the lack of visual evidence to support the basis of payment. Supplemental tests for mold, sediment, and acidity often are used for evaluating certain quality factors. However, the correlation between the results of these tests and organoleptic quality is lower than is desired. Furthermore, with the exception of the rapid acidity test, the supplemental tests often are too time consuming for practical use in cream stations for establishing the grade of cream before purchase.

Tests for protein and fat decomposition, even in simplified forms, largely are limited to laboratory use. In addition, their individual relationship with organoleptic grade appears to be too limited for general acceptance as a single measure of quality. No single test would be expected to detect the many possible defects contributing to poor quality. Flavor and odor undoubtedly will remain the principal criteria. Nevertheless, in view of the desirability of a rapid test having high correlation with quality as measured organoleptically and which might be used by cream buyers, field workers and inspectors, data are presented on a method devised for this purpose. The procedure is an outgrowth of observations made during the testing of cream for mold by the Parsons' modification (3) of the Wildman methylene blue-borax method (4). In the latter test it was noted that high quality cream often produced a light colored mixture while poor cream usually produced a darker shade. This observation prompted the testing of various dyes, indicators, and reagents to develop a procedure in which the color obtained with cream would show a suitably close relationship with quality.

## MATERIALS AND PROCEDURE

In developing the procedure, the primary objectives were to attain accuracy and simplicity, and to utilize as far as possible facilities and equipment commonly available in cream stations or small plant laboratories. These requisites have governed the quantities of cream and reagents used.

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*Crystal violet solution.* After preliminary tests with various dyes and indicators, crystal violet was selected as the most suitable for the test. The most satisfactory concentration in water had an optical density of 0.136[3] after further dilution of 1:250 to facilitate reading. With the lot of dye[4] used in this work the required concentration was obtained by dissolving 0.5 g. in 1 l. of distilled water and adjusting by further slight dilution in accordance with optical density readings. Although the solubility of the dye in water at 26° C. is given as 1.68 per cent, some sedimentation occurs in the 0.05 per cent solution after prolonged standing. It should be agitated before use and for obtaining consistent results in photometer readings. The dye solution at the concentration used was reasonably stable at room temperature. When held stoppered in 100-ml. to 500-ml. quantities away from direct sunlight, it remained satisfactory for use over periods up to 3 months.

*Sodium hydroxide.* Exploratory trials with the separate reagents used in the methylene blue-borax test and with several other bases showed that the color differences obtained with 0.1 *N* sodium hydroxide were the most closely associated with cream quality variations. This reagent accordingly was used in subsequent work. When kept stoppered, the 0.1 *N* sodium hydroxide remained of satisfactory concentration over a 6- to 8-week period. Since weaker base results in darker tests, occasional checking against 0.1 *N* hydrochloric acid is desirable if there is doubt as to the concentration of the sodium hydroxide.

*Color standard.* A color standard was used to indicate the line of demarkation between first and second grade cream. It was prepared to have the same color value as the majority of tests on cream of borderline quality (between first grade and second grade). Under the conditions of the test, and in accordance with the cream grade standards generally accepted in the Kansas area, this color value was near that commonly termed "Iris". Under a daylight-type fluorescent light it was similar to the color value designated as 43–6B in the "Dictionary of Color" (2). The color was reproduced by experimental mixing of white, red, and blue artists' oil paints. It then was applied to the outside of the lower half of a test tube (outside diameter 15 mm). Several tubes were prepared with slight variations in shade and intensity, so that, when dry, the one most closely representing the desired color under a daylight-type fluorescent lamp could be selected. For protection of the dried paint the tube was inserted into a larger test tube (inside diameter 16 mm.) and held in place with a cotton plug and

[3] A Coleman spectrophotometer, model 11, and a 0.5-cm. absorption cell were used. Readings were made at 580 mμ, at which setting the dye exhibited maximum absorption with this instrument.

[4] Distributed by Coleman and Bell Company, Norwood, Ohio. Cert. No. CC 10. Dye content 89 per cent.

cork stopper. The standard was kept in a pasteboard tube when not in use to minimize the possibility of fading. A 2-oz. sample jar painted on the lower half of the inside also was used occasionally as a standard.

*Comparator box.* Experimentation showed that comparisons of color value could be carried out best by using a small box, open at the front, as shown in figures 1 and 2. A hole was made in the center of the top side for the color standard tube. The box was made large enough to accommodate a 2-oz. sample jar at each side of the color standard. A blue background intensified differences in shade and gave more saisfactory results than the neutral gray usually recommended for color matching. Color

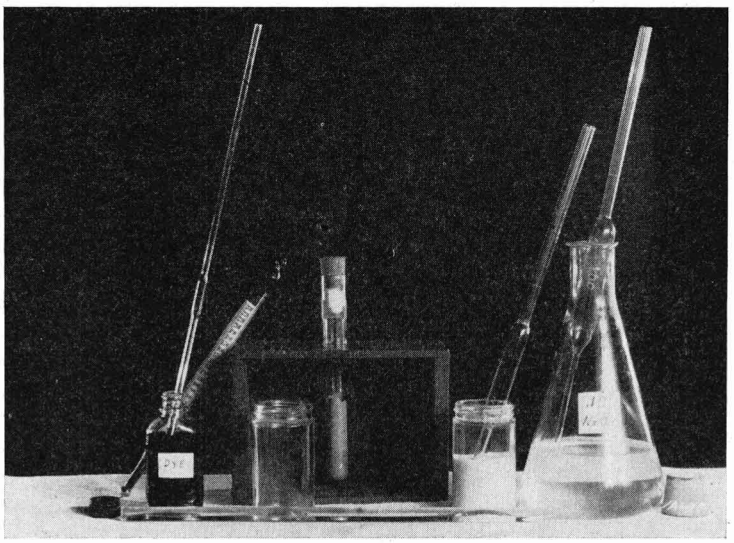

FIG. 1. Materials used for grading cream by the color test.

comparisons on tests were made by placing the box with tests so that light would shine in without shadows.

*Light source.* The light used for reading the tests affects the color comparison. Apparent changes in color as a result of different types of illumination are more marked in the tests than in the color standard. Since there is considerable variation in the intensity and the color of light in cream stations and other places where cream is graded, it is desirable to have a source of uniform light that will show consistent colors and emphasize small differences. As with color matching generally, a clear north sky light usually is satisfactory. Where this is not available conveniently, a 15- or 20-watt daylight-type fluorescent lamp which gives a slightly bluish light is desirable.

*Glassware* (fig. 1). The remaining equipment used for making the

test includes the following: 2-oz. cream sample jars (tall type), 1-ml. pipette, 9-ml. pipette, 17.6-ml. pipette, stirring rod, thermometer, bottles for solutions.

*Procedure for making the test.* Warm sample at about 100° F. until fluid and mix as for Babcock test, pipette 9 ml. of cream into a 2-oz. sample jar, add 17.6 ml. warm 0.1 *N* NaOH (about 120° F.), stir, add 1 ml. crystal violet dye solution and stir. Compare the test sample with color standard in comparator box under daylight-type fluorescent light or clear north sky light. Since the color fades fairly rapidly with cream of good quality, the tests should be read within 1 to 2 minutes after addition of the dye.

*Interpretation.* As demonstrated later, the color value obtained in the

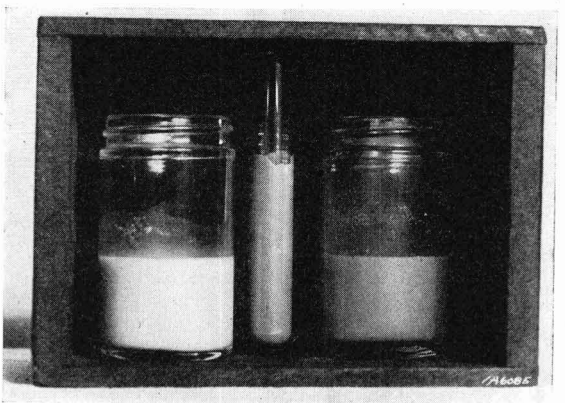

FIG. 2. The color test on first grade cream (left) and second grade cream (right) showing comparisons with color standard. Color differences are more obvious in the actual tests than in the black and white illustration.

test is primarily a function of both the acidity and the physical condition of the cream. It is based on the fact that crystal violet gradually is decolorized at pH values above 10.5 and that the depth of color obtained on the addition of dye to cream is influenced by the dispersion of cream constituents, more homogeneous distribution giving lighter shades. As cream deteriorates, such recognized changes as coagulation of casein, concentration of fat, separation of serum and other probable physico-chemical changes result in a less homogeneous medium than fresh cream and cause a deeper color with added dye. The physical change is augmented by the stirring and agitation the cream receives during accumulation under practical conditions of production.

With few exceptions the shade (lightness or darkness) of the test varies with the quality of the cream, being a light color with good cream and considerably darker with poor cream (fig. 2). Since the color standard

was prepared to have the same value as tests on cream of borderline quality (between first grade and second grade), those tests lighter than the standard are first grade and the tests darker than the standard are second grade or lower. The darkness or lightness of the color also indicates whether the quality corresponds with the upper or lower range of each grade. Tests with the same color as the standard indicate cream of borderline quality. Because an insufficient number of commercial cream samples of reject quality were obtained to establish a color value for this type of cream, such a color standard was not prepared. Even without this standard, experience in using the test would enable the operator to decide on the color value associated with unacceptable cream. When a variety of commercial reject quality cream samples could be obtained for comparative purposes, the necessary color standard could be prepared.

*Comparison of color tests with organoleptic grades.* The test was used on 780 samples of cream during spring, summer, fall and early winter.

TABLE 1

*Variations between organoleptic grades of commercial cream judged by individual graders in pairs*[a]

| Series[b] | Total samples graded | Whole grade variations | | Borderline variations resulting in different grades | | Total grade variations | |
|---|---|---|---|---|---|---|---|
| | (*no.*) | (*no.*) | (%) | (*no.*) | (%) | (*no.*) | (%) |
| 1 | 85 | 8 | 9.4 | 10 | 11.8 | 18 | 21.2 |
| 2 | 147 | 16 | 10.9 | 8 | 5.4 | 24 | 16.3 |
| 3 | 108 | 7 | 6.5 | 18 | 16.7 | 25 | 23.2 |
| Summary | 340 | 31 | 9.1 | 36 | 10.6 | 67 | 19.7 |

[a] Of the two graders for each series, one was the same in all series.
[b] Each series represents a different set of samples and a different pair of graders.

Most of the samples represented commercial cream as received at cream stations and creameries. The remainder were experimental samples. The result of the test on each sample was compared with the organoleptic grade. Most of the samples were graded by pairs of graders, with each individual working independently. The remainder of the samples were graded by one grader.

## RESULTS

*Variation between graders when using organoleptic methods.* In order to judge the value of the color test for grading purposes, it seemed desirable to have some information regarding the extent of variation usually prevailing between experienced graders on the same cream when grading by flavor and odor. Table 1 gives the results obtained with three series of samples of commercial cream, with two graders for each series, one of whom was the same in all series.

The results show there was disagreement in grades (including borderline differences) in 16.3 to 23.2 per cent of the cases, or an average of 19.7 per cent. Since, under the conditions involved, the graders undoubtedly graded more carefully than they would have done otherwise, this variation probably is less than would be obtained under practical operating conditions. From general observations it is believed that, with graders who have not worked together previously, agreement on grades of commercial cream probably would not be greater than 80 per cent. Accordingly, it is evident that the organoleptic grade of cream is not an absolute measure of quality. It is not an ideal standard by which to measure a proposed test, since differences may be due to inaccuracies in either method. Nevertheless, the organoleptic method is still the most practical, comprehensive and generally-accepted method of indicating cream quality, and state grade definitions are based largely on such an examination. Therefore, it was used as a standard in evaluating the color test described.

*Agreement of color test with organoleptic grade.* In the 780 samples of cream graded by both the described color test and by organoleptic means, the two methods agreed in 693 or 88.8 per cent of the cases. This compares with the approximately 80 per cent agreement obtained between human graders. Of the 11.2 per cent of the cases where there was disagreement between the color grade and the organoleptic grade, 77 (9.9 per cent of the total) were borderline differences as between a low first grade and a high second grade. Due to the human factors involved in the organoleptic method, there is some question as to whether these borderline differences really indicate inaccuracy of the color test. In only 1.3 per cent of the samples was there disagreement to the extent of a full grade, whereas experienced graders disagreed by a full grade on an average of 9.1 per cent of the samples graded (table 1).

Of the 780 samples which were graded either as first or second grade, 171 or 21.9 per cent were graded as second grade by the organoleptic method while 159 or 20.4 per cent were graded as second grade by the color test method. The color test placed 12 fewer samples (1.5 per cent) in the second grade than did the organoleptic method. This difference, however, is not significant, as indicated by a chi-square test which gave a value of 0.553 with one degree of freedom.

*Consistency of the test.* In order to determine if the test gave consistent results under similar conditions, 24 different samples of cream were tested in triplicate and comparisons made of the color of the three tests from each sample. No difference could be detected among the triplicates on any of the samples, indicating that the test gave consistent results when conditions were similar. This supported many general observations made during experimental work.

*Agreement in reading of color test by different individuals.* In order

to determine whether or not differences between individuals in reading the color test would result in significant differences in cream grading, samples from 30 different lots of cream delivered to cream stations by producers were tested. They then were read independently by three individuals using the same light source. Two of the judges had no previous experience in reading the test. The tests were read as lighter, darker, or the same as the standard, corresponding to first grade, second grade, and borderline quality cream. A chi-square test devised by Friedman (1) for ranked data was used to test the agreement among the three judges on the 30 samples. The chi-square was 1.52 with two degrees of freedom; hence it was concluded that the agreement among these individuals in reading the test was entirely satisfactory. Accordingly, since the procedure involved is simple, it is considered that the test is applicable for cream grading, even by inexperienced individuals.

### *Factors Determining the Color Value Obtained in the Test*

As previously stated, the color value obtained in the test is governed principally by the acidity and physical condition of the cream.

*Cream acidity.* Although crystal violet is not usually considered to be an indicator, it is decolorized at pH values above 10.5. The change is slow at values between 11 and 12 but is more rapid with increasing alkalinity. Accordingly, in the test as applied to cream, one of the principal factors governing the depth of color obtained is the excess alkalinity after the addition of the NaOH, which is influenced by the acidity of the cream. With cream that is almost sweet (0.2–0.3 per cent titratable acidity), the color of the test is light at the start and fades relatively fast. With high acid cream (either from added lactic acid or natural development) the color is much darker and fading is slower. The color differences between the pale hue obtained with fairly sweet cream and the dark shade obtained with high acid cream correspond to a wide range of alkalinity in the tests and generally represent a titratable acidity range in cream as wide as usually is encountered under practical conditions. Hence the test is a partial measure of cream acidity.

*Physical condition of the cream.* The influence of the physical condition of the cream was demonstrated by the fact that partially churned cream gave darker color tests than the same cream before agitation. In other trials occasional stirring of cream during holding in the laboratory resulted in darker tests than when no stirring was used, even though the final acidity of the stirred and the unstirred cream was practically the same. The effect of the physical condition was further illustrated by using a laboratory hand homogenizer to redisperse the constituents in old, low quality cream. Such treatment presumably resulted in a more homogeneous medium, similar to fairly fresh, good quality cream. The results of

six trials with sweet cream, sweet cream plus lactic acid, and old, low quality cream are given in table 2.

With sweet cream, homogenization gave no observable difference in the color test after most of the air incorporated had an opportunity to escape. When lactic acid was added to the sweet cream, the color test was darker. Homogenization of the cream with added acid caused only a slightly lighter color. This would be expected where the depth of the color was largely the result of acidity rather than of various physical changes in the cream. With second grade cream, however, the situation was different. Homogenizing the cream produced a much lighter color than was obtained in the test on the unhomogenized cream. This would indicate that the depth of

TABLE 2

*Effect of homogenizing the cream (in NaOH) on the color grade*

| Sample no. | Description of cream | Titratable acidity | Color grade | |
|---|---|---|---|---|
| | | | Homogenized[a] | Not homogenized |
| | | (%) | | |
| 1 | Sweet | 0.23 | 1 +[b] | 1 + |
| 2 | Sweet | ..... | 1 + | 1 + |
| 3 | Sweet + lactic acid | 0.70 | 1 | 1 |
| 4 | Sweet + lactic acid | 0.79 | 1 − | 1 − |
| 5 | Second grade cream | 1.10 | 1 − | 2 − |
| 6 | Second grade cream | ..... | 1 | 2 |

[a] In homogenizing, air is incorporated and influences the color somewhat. Hence color comparisons were made after most of the air had an opportunity to escape.

[b] + indicates upper range of grade and − indicates lower range of grade.

color was influenced partly by acidity and partly by physical dispersion of the cream constituents.

Although the color obtained in the test is related independently to both the acidity and physical condition of the cream, the combined effect of these two factors gives results more closely related to organoleptic quality than is produced by either factor alone. The fact that the influence of acidity sometimes is modified by the influence of physical condition and vice versa apparently is the reason for the relationship under practical conditions. Examples are presented in table 3 to show that although the color grade generally is associated with acidity, there are exceptions. These exceptions particularly are evident in samples of high acid cream that were of clean flavor and smooth texture. Such samples were of higher organoleptic quality and showed higher color grades than indicated by titratable acidity. On the other hand, some cream samples of lower acidity were also of low quality as shown by both organoleptic tests and the color tests. Such variations between organoleptic grade and cream acidity generally are recognized, and in this respect the color test is in accord with the organoleptic method.

Many other observations made while using the test indicated that the physical condition of the cream (probably associated with the physico-chemical condition) modified the color obtained in the test. Smooth, clean, high-acid cream often graded higher by the test than did other cream of lower acidity but which was grainy, curdled, partially churned, or had other physical characteristics usually associated with low quality cream. In this characteristic the test agrees with recommended grading practices.

*Fat content of cream.* Tests on sweet cream of 35 to 40 per cent fat content showed very little difference in color from tests on the same cream diluted to 20 to 25 per cent fat with skim milk. The same dilution with water caused a slightly darker test. Apparently variations in fat content

TABLE 3

*Variations between titratable acidity and grade of cream*

| Sample no. | Titratable acidity | Color grade | Organoleptic grade | Remarks |
|---|---|---|---|---|
| 1 | 0.20 | 1 +[a] | 1 + | ..... |
| 2 | 0.51 | 1 + | 1 + | ..... |
| 3 | 0.53 | 1 | 1 | ..... |
| 4 | 0.53 | 1 – | 1 – | ..... |
| 5 | 0.61 | 2 + | 1 – | Thin, watery |
| 6 | 0.65 | 1 | 1 | ..... |
| 7 | 0.68 | 1 + | 1 | Clean |
| 8 | 0.73 | 1 – | 1 | ..... |
| 9 | 0.73 | 2 | 1 – | ..... |
| 10 | 0.91 | 2 + | 2 | ..... |
| 11 | 1.08 | 2 + | 2 + | ..... |
| 12 | 1.10 | 2 – | 2 | ..... |
| 13 | 1.15 | 1 – | 1 – | Clean, smooth, high acid |
| 14 | 1.15 | 2 + | 2 | ..... |
| 15 | 1.20 | 1 – | 1 – | Clean, smooth, high acid |
| 16 | 1.24 | 2 | 2 – | ..... |

[a] + indicates upper range of grade and – indicates lower range of grade.

within the range commonly encountered under practical conditions have only a minor influence on the color obtained in the test. That the depth of color obtained is not dependent primarily on the fat content also is shown by the fact that lots of the same cream will give different color tests when held under different conditions resulting in quality variations.

*Amount of cream in sample.* Even with warm fluid cream there is some variation in the amount of cream measured from different samples. Observations where weighed samples were compared with measured samples and where measured amounts were varied by 0.5 ml. showed that such differences in size of the sample had little influence on the color obtained in the test.

*Color and thickness of glass in sample jars.* Comparisons indicated that differences in the common 2-oz. sample jars did not cause observable differences in the apparent color of the test.

## DISCUSSION

It is unlikely that organoleptic grading of cream, where carefully applied, will be satisfactorily replaced by other tests. However, in circumstances where it has been difficult to promote cream grading, where quality is questionable and where such grading is most needed, it is evident that some simple cream grading aid is desirable. It is considered that the test described has merits in this respect. Although the test was studied under Kansas conditions and standardized to the quality standards and grades in that state, it easily could be adapted to conditions existing in most of the Middle-west area where cream stations are common.

With the exception of the color standard, the test utilizes simple, readily available equipment and is rapid and easy to operate. Although laboratory facilities are necessary for their preparation, the reagents are sufficiently stable for use over a period of several months if kept stoppered. Accordingly, if the reagents and equipment were assembled in the form of a field kit, subsequent operation of the test in stations or factories would be simple.

With few exceptions, the test showed a marked difference in color between good and poor cream. In areas and during seasons when the main cause of low cream quality is deterioration, the test should be useful for grading and in visually demonstrating to cream buyers and producers the different quality grades of cream. The fact that the color value obtained in the test, although influenced by acidity, also is modified by the physical condition of the cream is advantageous. Under practical conditions of production and marketing farm-separated cream, there seems to be a close relationship between the age and quality of cream and its physical condition. Presumably this is due partly to the fact that the longer it takes to accumulate, the more stirring or agitation the cream receives. Also, with increase in age and acidity, the physical or physico-chemical condition undoubtedly is modified, as evidenced by easier churning or whipping. Even at fairly low temperatures there is some separation of fat and serum. Although such changes may not always be evident to the eye, they sometimes are emphasized by the tendency of the cream to oil off on the addition of hot water. General recognition of the relationship between quality and physical condition of cream is indicated in the cream grading laws of several states, which stipulate that first grade cream must be smooth and free from lumps, and that lumpy, curdy cream is second grade. The characteristic of the color test of grading down the latter type of cream is in accordance with recommended grading practices.

Where state cream grade definitions place an acidity limit on grades, it would seem that a rapid acid test often would be a necessary complementary test to any other grading method. Although the described color test correlates fairly well with acidity, and the color standard could be modified to compare with desired acidity limits, other factors associated

with quality also are measured by the test. In areas where cream quality generally is high and deterioration is a relatively minor factor compared with other flavor defects (absorbed, weed, feed, etc.), the test would be expected to show less correlation with quality measured organoleptically.

## SUMMARY AND CONCLUSIONS

1. A simple color test as an aid in grading farm-separated cream at time of purchase is described. Instructions for the preparation of reagents, description of equipment needed and details for the testing procedure are given.

2. Although the preparation of reagents and the color standard requires laboratory facilities, the operation of the test is simple and is particularly suitable for field work and cream station conditions.

3. The test is based on the depth of color resulting from the addition of 17.6 ml. of 0.1 *N* NaOH and 1 ml. of crystal violet dye solution of given concentration to 9 ml. of cream in a 2-oz. sample jar. Under the conditions of making the test, the color value obtained primarily is related to the acidity and the physical condition of the cream. Under practical conditions of production the combined acidity and physical condition of cream appear to correlate closely with organoleptic grade.

4. Comparison with a prepared color standard of the color value obtained on cream tests permits cream to be graded as first or second grade, and also usually indicates whether it falls in the upper or lower range of the grade.

5. The test was used on 780 samples of experimental and commercial cream from stations and creameries during late spring, summer, fall and early winter. Agreement with organoleptic grades was obtained to the extent of 88.8 per cent of the cases. Most of the differences were only borderline variations. In only 1.3 per cent of the samples did the difference represent a whole grade. This was closer agreement than was obtained between organoleptic grades as judged by experienced graders.

6. It is expected that the test would be most applicable in those areas where poor cream quality is largely the result of deterioration rather than of flavor defects of other types. Since the test is a partial measure of acidity, the color standard could be modified so that the test would be useful even in states where specific acidity limits are placed on cream grades.

7. From the results obtained it appeared that the test offers a means of promoting cream grading in localities where little grading is practiced and where general improvement in quality is needed.

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# THE EFFECT OF PREPARATION OF THE COW ON THE RATE OF MILKING[1, 2]

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One of the important jobs in the management of a dairy herd is that of milking. Several workers have reported on various aspects dealing with this problem. Studies by Zwart (11) and Tgetgel (10) showed a gradual rise in udder pressure from one milking to the next. Gaines (6), Tgetgel (10) and Krzywanek and Brüggemann (8) noted marked increases of intraglandular pressure after mammary stimulation.

Gaines (6, 7), Espe (4) and Foot (5) observed latent periods of varying lengths after stimulation before a "let-down" or excretion of milk occurred.

Elting and LaMaster (2) studied the effect of foremilking on the rate of mechanical milking. They reported that foremilking increased the rate of milk flow in the earlier part of the milking process but prolonged the time required for stripping. The over-all effect was an increase in the total time required for milking. Dodd and Foot (1) found that stimulation before milking shortened the time required for milking. The object of this experiment was to ascertain the effect of preparation or obtaining a let-down of milk before attachment of the milking machine on the rate of milk withdrawal.

## EXPERIMENTAL PROCEDURE

Four 2-year-old Holsteins, E401, E405, E413 and A55, and one of mixed breeding, E408, and a 5-year-old grade Holstein, A30, were used in this study. Cows E401 and E405 were hand milked before the beginning of this experiment. Cows E408, E413 and A55 were milked by machine beginning 3 days after calving. A30 was in her third lactation and had been milked by machine in previous lactations.

During the course of the experiment the cows were milked twice daily at 12-hour intervals. At the evening milking of one day and the morning milking of the following day, the cows were stimulated before milking. At the successive evening and morning milking the cows were not stimulated previous to milking. The cows were stimulated to let down or excrete

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milk by a 10- to 15-second wash and massage of the teats and udder with water at a temperature between 120 and 130° F. 2 minutes before the milking process began. Before attaching the teat cups, each quarter was foremilked by expressing two streams of milk from each teat. For the non-stimulated milkings, the teat cups were merely attached without prior washing or massaging of the teats or udder.

The milking machine was suspended from a scale and readings were taken every 10 seconds from the time the last teat cup was put on until the "end-point" of milking was reached. For the purposes of this study the end-point of a milking was taken as the time after milking when the increment in yield of three successive 10-second scale readings was three-

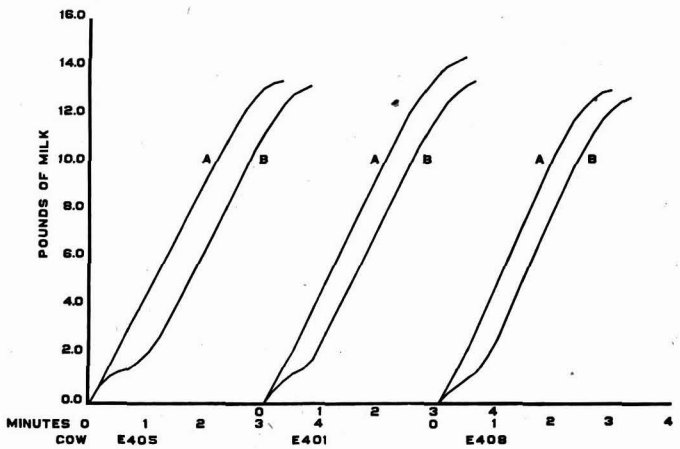


FIG. 1. *The effect of stimulation and non-stimulation on the shape of the milk curve.* A—Stimulated; B—Not stimulated.

tenths of a pound or less, or when two successive readings were identical. The criterion used depended on which occurred first. The use of an end-point was justified by the fact that preliminary readings had shown that the rate of flow was practically constant to the end-point and machine stripping should occur at that stage of the milking process. A stop watch was used for timing purposes. Ten milkings were recorded for each régime. The milking machine used was a double-action, constant-vacuum type and was operated at 15 inches of mercury negative pressure, commonly called vacuum, on the line and 50 pulsations per minute. There was a difference of 0.5-inch mercury negative pressure between the line and milk hose when milk was not flowing.

## RESULTS

Table 1 presents the results of this study. Figure 1 shows the milk curve for three of the cows. A lapse of 30 to 60 seconds occurs before the

TABLE 1

*Mean accumulative total pounds of milk for ten milkings when the cows were stimulated and not stimulated before attaching the milking machine*

| Milking time (minutes and seconds) | Cow E405 | | Cow E401 | | Cow E408 | | Cow A30 | | Cow A55 | | Cow E413 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Stimulated | Not stimulated | Stimulated | Not stimulated | Stimulated | Not stimulated | Stimulated | Not stimulated | Stimulated | Not stimulated | Stimulated | Not stimulated |
| | (*lb.*) | (*lb.*) | (*lb.*) | (*lb.*) | (*lb.*) | (*lb.*) | (*lb.*) | (*lb.*) | (*lb.*) | (*lb.*) | (*lb.*) | (*lb.*) |
| 0: 10 | 0.71 | 0.65 | 0.71 | 0.48 | 0.65 | 0.41 | 0.98 | 0.56 | 0.58 | 0.32 | 0.78 | 0.53 |
| 0: 20 | 1.52 | 1.08 | 1.46 | 0.86 | 1.39 | 0.73 | 1.75 | 0.77 | 1.21 | 0.70 | 1.55 | 1.16 |
| 0: 30 | 2.33 | 1.29 | 2.21 | 1.15 | 2.22 | 0.98 | 2.52 | 0.88 | 1.85 | 1.09 | 2.43 | 1.66 |
| 0: 40 | 3.18 | 1.43 | 3.03 | 1.42 | 3.08 | 1.30 | 3.38 | 1.10 | 2.49 | 1.47 | 3.28 | 2.09 |
| 0: 50 | 3.95 | 1.65 | 3.88 | 1.83 | 3.99 | 1.90 | 4.20 | 1.62 | 3.15 | 2.03 | 4.16 | 2.77 |
| 1: 00 | 4.73 | 2.08 | 4.71 | 2.56 | 4.89 | 2.64 | 5.04 | 2.36 | 3.84 | 2.63 | 5.06 | 3.55 |
| 1: 10 | 5.51 | 2.70 | 5.53 | 3.36 | 5.82 | 3.52 | 5.89 | 3.23 | 4.53 | 3.28 | 6.00 | 4.46 |
| 1: 20 | 6.28 | 3.43 | 6.38 | 4.18 | 6.75 | 4.41 | 6.74 | 4.12 | 5.21 | 3.93 | 6.89 | 5.30 |
| 1: 30 | 7.04 | 4.20 | 7.21 | 5.01 | 7.70 | 5.36 | 7.63 | 5.03 | 5.88 | 4.61 | 7.81 | 6.19 |
| 1: 40 | 7.86 | 4.98 | 7.98 | 5.84 | 8.65 | 6.29 | 8.48 | 5.91 | 6.54 | 5.23 | 8.65 | 7.03 |
| 1: 50 | 8.63 | 5.79 | 8.90 | 6.63 | 9.52 | 7.19 | 9.30 | 6.78 | 7.16 | 5.79 | 9.46 | 7.84 |
| 2: 00 | 9.40 | 6.54 | 9.68 | 7.45 | 10.36 | 8.10 | 10.15 | 7.64 | 7.70 | 6.52 | 10.19 | 8.68 |
| 2: 10 | 10.18 | 7.38 | 10.52 | 8.27 | 11.08 | 9.02 | 10.94 | 8.53 | 8.42 | 7.17 | 10.94 | 9.47 |
| 2: 20 | 10.94 | 8.14 | 11.33 | 9.09 | 11.75 | 9.86 | 11.65 | 9.43 | 9.02 | 7.82 | 11.67 | 10.15 |
| 2: 30 | 11.64 | 8.95 | 12.08 | 9.93 | 12.32 | 10.64 | 12.34 | 10.28 | 9.62 | 8.42 | 12.43 | 10.87 |
| 2: 40 | 12.27 | 9.79 | 12.69 | 10.74 | 12.74 | 11.32 | 12.93 | 11.06 | 10.15 | 9.07 | 13.15 | 11.57 |
| 2: 50 | 12.77 | 10.57 | 13.22 | 11.35 | 12.99 | 11.87 | 13.45 | 11.77 | 10.60 | 9.69 | 13.77 | 12.29 |
| 3: 00 | 13.17 | 11.30 | 13.69 | 12.04 | 13.12 | 12.31 | 13.95 | 12.44 | 10.97 | 10.21 | 14.24 | 12.97 |
| 3: 10 | 13.44 | 11.91 | 14.06 | 12.60 | ..... | 12.59 | 14.33 | 13.02 | 11.28 | 10.71 | 14.53 | 13.60 |
| 3: 20 | 13.62 | 12.46 | 14.33 | 13.03 | ..... | 12.75 | 14.69 | 13.55 | 11.54 | 11.16 | 14.71 | 14.14 |
| 3: 30 | ..... | 12.87 | 14.50 | 13.32 | ..... | ..... | 14.87 | 14.02 | 11.71 | 11.57 | ..... | 14.51 |
| 3: 40 | ..... | 13.10 | ..... | 13.46 | ..... | ..... | ..... | 14.37 | ..... | 11.89 | ..... | 14.70 |
| 3: 50 | ..... | 13.27 | ..... | ..... | ..... | ..... | ..... | 14.70 | ..... | 12.13 | ..... | 14.82 |
| 4: 00 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 14.85 | ..... | 12.29 | ..... | ..... |
| Total milk obtained | 14.63 | 14.76 | 14.97 | 15.03 | 14.53 | 14.53 | 16.58 | 16.16 | 13.05 | 13.40 | 15.67 | 15.95 |
| Per cent total at end point | 93.1 | 89.9 | 96.9 | 89.6 | 90.3 | 87.7 | 89.7 | 91.9 | 89.7 | 91.7 | 93.9 | 92.9 |
| Mean rates per min. | 4.08 | 3.48 | 4.14 | 3.66 | 4.38 | 3.84 | 4.26 | 3.72 | 3.36 | 3.06 | 4.44 | 3.72 |

milk is let down when the cows are not stimulated, as evidenced by examination of the table and the plateaus in the curves. The curves of the rate of milk removal are markedly different in shape when the cows were stimulated and not stimulated before attaching the milking machine.

All six cows had appreciably higher mean rates of removal of milk when stimulated before milking. The rates of milk flow after stimulation for cows A30, A55, E413, E405, E401 and E408 were 0.71, 0.56, 0.74, 0.68, 0.69 and 0.73 lb. per 10 seconds, respectively, whereas the rates for non-stimulation of the cows in the same order were 0.62, 0.51, 0.62, 0.58, 0.61 and 0.64 lb. per 10 seconds. From 10 to 30 seconds less time was required to reach the end-point when the cows were stimulated, and only with cows A30 and A55 was the percentage of the total at the end-point slightly lower than when not stimulated.

A30 was milked for a period of 15 consecutive days to test the response to continuous non-stimulation. The milking machine was operated under the same conditions as in the previous experiment. The mean total at 1 minute for 29 milkings (readings for one milking were missed when the milk hose dropped off) was 2.38 lb. Reference to table 1 shows that the mean total of ten milkings at one minute was 5.04 lb. for A30 when stimulated before milking. The mean total at 1 minute of the continuous non-stimulation milkings was very similar to that of the mean of the ten milkings, 2.36 lb. of non-stimulation when alternated daily with stimulation before milking.

## DISCUSSION

A very definite plateau occurred in the milk curves when the cows were not stimulated, as the milk had not been let down and the sinuses were soon evacuated. The results obtained in this investigation are in agreement with the findings of Gaines (6, 7), Foot (5) and Espe (4). Practically a constant rate of flow was obtained from the time the milking process began until the end-point was reached when the cows were prepared for milking by prior stimulation. The initial flow of milk of about a pound when the cows were not stimulated represents the milk that had drained into the large ducts and the gland and teat sinuses.

Ely and Petersen (3) noted a response by the let-down or excretion of milk 45 seconds after the injection of oxytocin. Thus, the plateau in the milk flow curve of cows not prepared for milking by stimulation is the time necessary for the milking stimulus to motivate the posterior lobe of the pituitary to secrete the oxytocic principle into the blood stream and cause a let-down or excretion of milk.

Before stimulation, the teats are soft and flabby, but they become firm and turgid after stimulation as a result of the let-down or excretion of milk, with a resulting increase in intraglandular pressure. It was observed that teat cups are much more easily attached to a turgid than a flabby teat.

Petersen (9) reported that the teat cups crawl or draw in the slackened udder tissue when the intraglandular pressure is low and occlude the orifices between the gland and teat sinuses. The results of this study show that the time required for milking was longer when the cows were not stimulated. Obviously a milking machine attached to the teats of an udder in which an increment of intraglandular pressure has not been effected by stimulation may occlude the passage between the gland and teat at the beginning of milking, thereby prolonging the milking process. In addition, when the sinuses have been drained, as represented by the plateau in the milking curves, the teat cups draw in the flaccid udder tissue and trauma may result to the secretory tissue at the juncture of the teat and gland.

## SUMMARY AND CONCLUSIONS

Preparing the cow for milking by stimulation with a wash and massage of the udder with water at 120 to 130° F. was found to increase the rate of milking and decrease the time required for the milking process as compared with no preparation.

A grant from Babson Brothers Milker Company, Chicago, Illinois, that made this study possible is gratefully acknowledged.

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# THE REDUCING CAPACITY OF MILK AND MILK PRODUCTS AS MEASURED BY A MODIFIED FERRICYANIDE METHOD[1,2]

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In 1945 Chapman and McFarlane (5) published a method for determining reducing substances in milk and milk products by heating with potassium ferricyanide under specific conditions. The method was adapted from the procedure used by Anson (1, 2), Mirsky (12) and Mirsky and Anson (13) for determining sulfhydryl groups in proteins. Chapman and McFarlane found that fresh milk possesses considerable capacity to reduce ferricyanide under the conditions used and that heat treatment of milk or storage of milk powder open to the atmosphere increases the reducing capacity.

In 1945 Harland and Ashworth (8) reported the use of thiamin disulfide for estimation of the reducing power of milk. This reagent evidently is a much weaker oxidant than ferricyanide, at least under the conditions used, since it is not reduced at all by normal unheated milk. Heat treatment, however, does produce materials which reduce thiamin disulfide but long continued heat treatment in the presence of air causes a subsequent decrease in reducing power. The disparity in behavior of milk to these two reagents prompted Chapman and McFarlane (6) to express the opinion that these reagents react with different reducing systems. This opinion is somewhat substantiated by the work of Lea (10), which indicates that materials produced by interaction of lactose and protein are responsible for the increase in reducing capacity of dry milk during storage at 47° C. and 55 per cent relative humidity.

The work reported in this paper was undertaken prior to publication of Lea's results to examine the ferricyanide method and to determine which constituents of milk reduce this reagent and contribute to the increases produced by processing and storage.

## METHOD

*Factors affecting reduction of ferricyanide.* The extent to which ferricyanide is reduced by a system such as milk is strongly influenced by the

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[3] Professor of Dairy Husbandry, University of Nebraska.

hydrogen ion concentration, which determines the reduction potential of the several reductants present. Furthermore, since the reaction is slow, it usually is not allowed to go to completion. Hence the temperature and time of the reaction become of great importance in determining the extent of reduction. Chapman and McFarlane (5) made some study of the effect of the three variables—pH, temperature, and time—on the amount of ferricyanide reduced by milk powder. They showed: (a) that the capacity to reduce ferricyanide increases markedly from pH 2 to pH 8, (b) that the reaction proceeds much faster at 70° C. than at 50° C. (at pH 5.0),

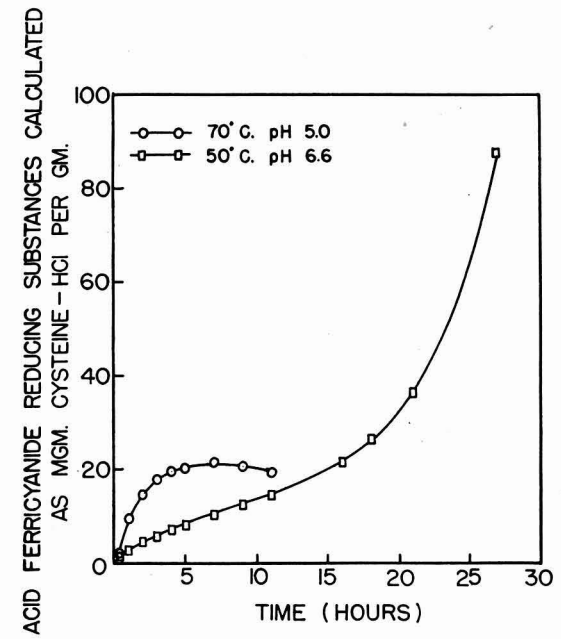


FIG. 1. Reduction of ferricyanide by dry whole milk.

and (c) that the reaction apparently is far from complete in 40 minutes (at 70° C. and pH 5.0). Others (13) have shown that protein continues to reduce ferricyanide for 12 to 24 hours at least. On the basis of their study of the factors influencing the reduction, Chapman and McFarlane (5) adopted the standard conditions of pH 5.0, 70° C., and 20 minutes as giving a satisfactory differentiation between fresh and aged samples.

In employing the method of Chapman and McFarlane, the present authors soon encountered a serious difficulty. In some cases, particularly with powders of high reducing capacity, a blue precipitate was retained on the filter paper when the reaction mixture was deproteinized. This phenomenon could be attributed to partial decomposition of the ferricyan-

ide during heating with liberation of ferric ions which react with ferrocyanide to form Prussian blue (ferric ferrocyanide). Since the extent of reduction is determined by formation of Prussian blue after deproteinization, any such removal of ferrocyanide by "preformation" of Prussian blue might seriously vitiate the results. Consequently, conditions which would obviate this difficulty were sought for conducting the reaction.

It soon was found that by raising the pH to 6.6 and lowering the temperature to 50° C., no Prussian blue was preformed, although the rate of

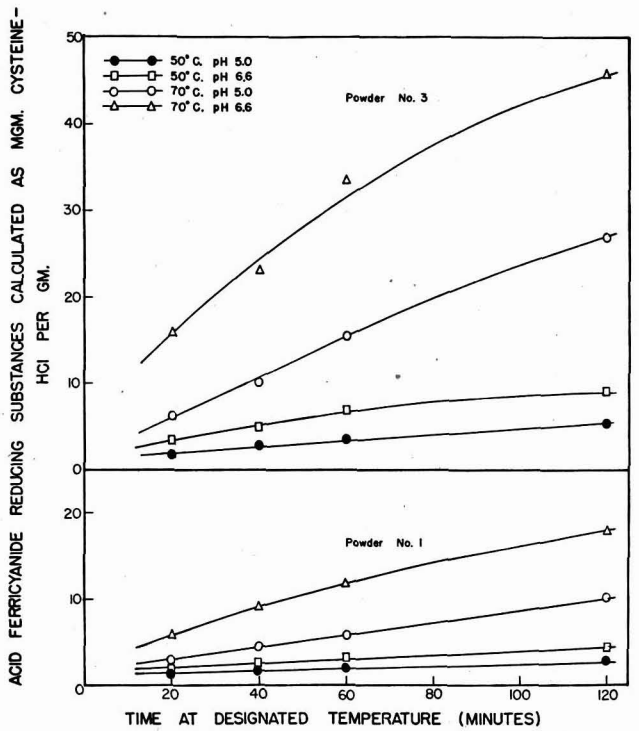


FIG. 2. Effect of temperature and pH on rate of reduction of ferricyanide by two samples of dry whole milk.

reduction was somewhat lower than that at pH 5.0 and 70° C. Figure 1 shows a comparison of the rate of reduction at pH 5.0, 70° C., with that at pH 6.6, 50° C. The fact that the reduction at pH 5.0, 70° C. reaches a maximum and even tends to drop off is attributable to the "preformation" of Prussian blue. Evidently, reduction continues indefinitely at pH 6.6, 50° C. Figure 2 shows the rates of reduction of two powders under the following conditions: pH 5.0, 70° C.; pH 5.0, 50° C.; pH 6.6, 70° C.; and pH 6.6, 50° C. Of these four, the last yielded satisfactory differentiation

without the use of excessively high temperature; consequently, it was adopted, together with a standard reaction time of 20 minutes.

Chapman and McFarlane (5) stated that the use of 5 ml. of 1 per cent ferricyanide per 100 mg. of milk powder yielded maximum color intensity. In the present study limited data indicate that greater intensities are obtained by increasing the concentration of ferricyanide. However, this point has not been investigated very extensively, and 5 ml. of 1 per cent solution have been employed routinely.

Folin (7) and Anson (1) have indicated that impurities may be encountered in ferricyanide and have suggested methods for purification. Furthermore, Anson (1) advised storage of ferricyanide at 5° C. in the dark and checking it occasionally for the presence of ferrocyanide. In the present study no evidence of impurities in reagent grade ferricyanide was observed, but solutions of it did deteriorate at room temperature. No evidence of deterioration of solutions stored in the dark at 5° C. for periods up to 15 days has been found.

*Factors affecting color intensity.* Chapman and McFarlane (5) stipulated the use of "freshly prepared" ferric chloride solution for formation of Prussian blue in the deproteinized filtrate. In the present study, holding such solutions for periods up to 4 days was practically without effect on color intensity, but the general rule of preparing fresh solution each day was adopted.

The procedure of Chapman and McFarlane (5) in reading the color intensity at exactly 10 minutes after addition of the ferric chloride solution was followed. Under these conditions, of course, the color intensity does not follow Beer's law and a calibration curve must be used. Lea (10) has reported that, if the holding period is limited to one minute, Beer's law is obeyed, but in the opinion of the present workers any advantage gained by such a procedure is offset by the fact that slight variations in holding time would introduce larger errors than in the case of the 10-minute holding period.

*The method adopted.* Weigh a 100-mg. sample of dry milk or simplified system into a test tube (22 × 150 mm.) and disperse it in 5 ml. of distilled water at 50° C. Alternatively reconstitute 5 g. in 250 ml. of distilled water and use a 5-ml. aliquot. Add 5 ml. of a buffer at pH 6.6 (M/5 potassium dihydrogen phosphate and M/5 sodium hydroxide) and 5 ml. of 1.0 per cent potassium ferricyanide. Mix well and heat for exactly 20 minutes in a continuously agitated water bath maintained at 50° C. Cool immediately to 25° C. or lower in an ice water bath. Add 5 ml. of 10 per cent solution of trichloracetic acid, mix and filter through no. 40 Whatman filter paper. Transfer 5 ml. of the filtrate to a test tube (22 × 150 mm.) and dilute with 5 ml. of distilled water. Add 1 ml. of fresh 0.1 per cent ferric chloride solution and mix thoroughly by vigorous shaking. If several de-

terminations are being made, add the ferric chloride solution to the tubes at intervals of 1 minute and hold each tube for exactly 10 minutes before determining the color intensity. A pair of matched square cuvettes and a Coleman Universal Spectrophotometer have been used, making all readings at 660 mμ with the reagent blank set to read 100 per cent transmission. The reagent blank is similar to the unknown except that 5 ml. of water is substituted for the sample.

In some cases it was desired to determine the proportion of the reducing capacity contributed by components of the system other than protein. For this purpose a portion of reconstituted sample was deproteinized with

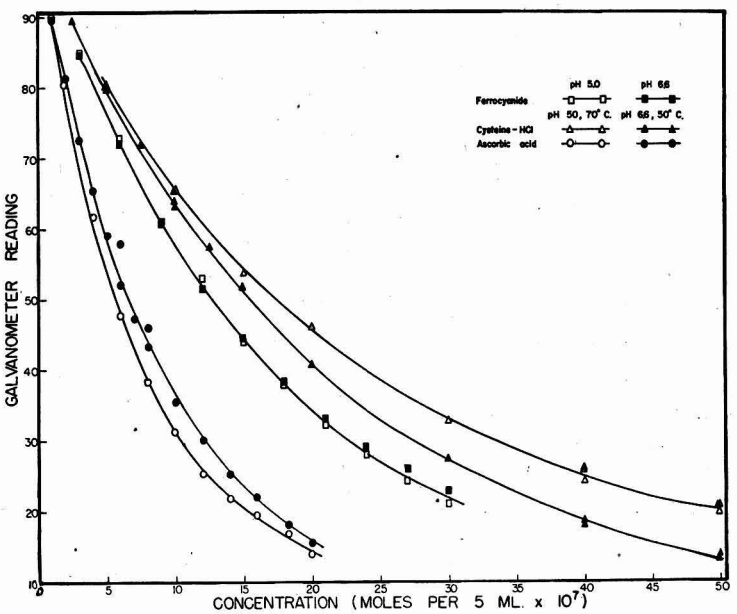


FIG. 3. Calibration curves showing the relation of the intensity of Prussian blue color to concentration of ferrocyanide, cysteine, and ascorbic acid.

tungstic acid and ferricyanide reduction determined in the filtrate. The use of a buffer having a pH of 7.4 was found necessary to insure a final pH of 6.6. The detailed procedure is as follows: Reconstitute 1 g. of powder with 25 ml. of distilled water in a 50-ml. volumetric flask. Add 16 ml. of 0.33 *N* sulfuric acid, 8 ml. of 10 per cent sodium tungstate and sufficient distilled water to bring to volume. Mix thoroughly, hold for 10 minutes and filter. For the ferricyanide reduction use 5 ml. of filtrate (equivalent to 100 mg. of powder) and 5 ml. of buffer at pH 7.4. Proceed from this point exactly as for whole milk except that filtration after adding trichloroacetic acid may be omitted.

*Expression and reproducibility of results.* The choice of units for expression of the reducing power of milk is complicated by the multiplicity of reductants involved. Faced with this situation, Chapman and McFarlane (5) chose to calibrate the method with glutathione and to express the capacity of milk to reduce ferricyanide in terms of the molar concentration of glutathione sulfhydryl groups required for an equivalent reduction. However, Lea (10) contends, with considerable justification, that, in view of the lack of knowledge of the specific groups involved, it is preferable to express reducing power of milk in terms of moles of ferricyanide reduced.

Figure 3 shows a curve relating galvanometer reading to concentration of potassium ferrocyanide. In obtaining the data for this curve a series of solutions containing 1.0 per cent ferricyanide and concentrations of ferrocyanide up to $60 \times 10^{-5}$ molar was prepared. Five milliliters of such solution, 5 ml. of buffer (either pH 5.0 or pH 6.6), 5 ml. of water, and 5 ml. of 10 per cent trichloracetic acid then were mixed and a 5-ml. aliquot taken. To this was added 5 ml. of water and 1 ml. of 0.1 per cent ferric chloride and the color intensity read after 10 minutes. Little if any effect of pH on color development was found. Figure 3 also shows curves relating concentration of cysteine and ascorbic acid to the intensity of blue color obtained from ferrocyanide produced by reduction of ferricyanide by these reductants.

In figure 4, the concentration of ferrocyanide necessary to produce a given color intensity has been plotted against the concentration of cysteine or ascorbic acid which produces an identical intensity by reduction of ferricyanide. Ascorbic acid reacts very nearly stoichiometrically at pH 6.6, 50° C. with ferricyanide; the slope of the line indicates that 1 mole of ascorbic acid reduces 1.95 moles of ferricyanide, which is very close to the theoretical equivalent of 2.00. At pH 5.0, 70° C., slightly more than 2 moles of ferricyanide are reduced by a mole of ascorbic acid. Tauber and Kleiner (14) employed a somewhat similar method for determining ascorbic acid but the reduction was carried out at a lower temperature (40° C.) in a more acid medium (10 per cent trichloracetic). Their data give no evidence as to the stoichiometry of the reaction. (See also Ball (3).)

The extent of reduction by cysteine is neither so complete nor so uniform over the concentration range studied as is that by ascorbic acid. In the range of concentration from 5 to $32 \times 10^{-7}$ moles per determination, a mole of cysteine reduced about 0.85 mole of ferricyanide at pH 6.6, 50° C., and about 0.70 mole at pH 5.0, 70° C. Mason (11) estimated glutathione by oxidation with ferricyanide at pH 5.9 at room temperature and determination of Prussian blue. The oxidations of cysteine and glutathione were stoichiometrically equivalent under these conditions, but the paper gives no information as to whether the ferricyanide reduced is stoichiometrically equivalent to the cysteine or glutathione oxidized. However,

Anson (1) obtained 0.00104 mM of ferrocyanide when 0.001 mM of cysteine was oxidized with 0.2 mM of ferricyanide for 20 minutes at pH 6.8, 50° C., which does indicate a stoichiometric relation. While these data support Lea's (10) contention that the reduction of ferricyanide by cysteine is not stoichiometric under the conditions used, they do furnish an empirical relation which could be used in converting from one basis to another.

All results are expressed in terms of equivalent cysteine hydrochloride

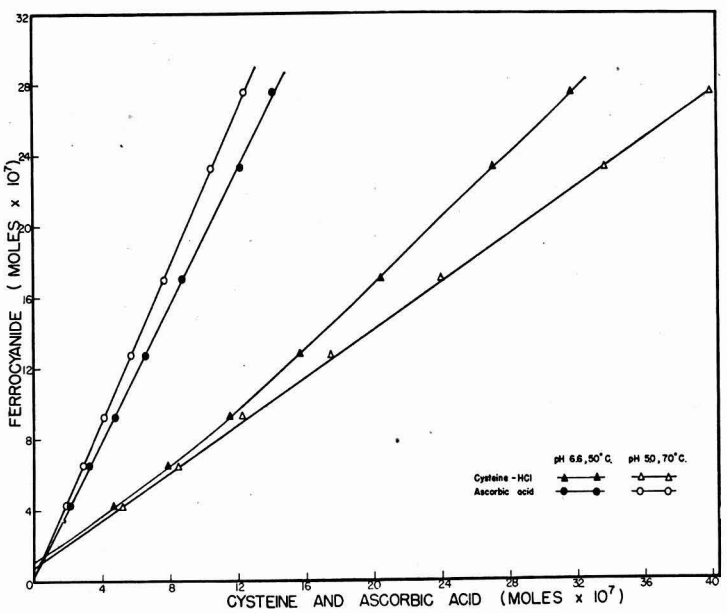


FIG. 4. Relation between cysteine or ascorbic acid oxidized and ferrocyanide produced.

concentrations because such standardization furnishes a common basis for comparing various methods.

To test the reproducibility of results, several 100-mg. samples of two different milk powders were weighed into test tubes which were stoppered and held at –10° C. On several occasions determinations were made on these samples by two individuals working independently. The results, shown in table 1, indicate a rather satisfactory degree of reproducibility for either individual. However, there is a small but consistent inexplainable difference between individuals.

## MATERIALS

The materials used in this study were those described in a recent paper on fluorescence (9). Briefly, they were spray dried milk, acid-precipitated

TABLE 1

*Comparison of results obtained by two individuals with the ferricyanide method*

| Trial[a] | Reducing substances as cysteine-HCl per g. | | | |
|---|---|---|---|---|
| | Sample 358 | | Sample 49 | |
| | A[b] | B[b] | A[b] | B[b] |
| | (*mg.*) | (*mg.*) | (*mg.*) | (*mg.*) |
| 1 | 1.32 | 1.42 | 2.88 | 2.93 |
| 2 | 1.32 | ..... | 2.84 | ..... |
| 3 | 1.36 | ..... | 2.95 | ..... |
| 4 | 1.38 | 1.41 | 2.84 | 3.01 |
| 5 | 1.37 | 1.45 | 2.84 | 2.92 |
| 6 | 1.32 | 1.41 | 2.90 | 2.87 |
| Mean | 1.35 | 1.42 | 2.88 | 2.93 |

[a] Each trial made on a different day.
[b] Individuals designated A and B.

casein, dialyzed milk serum protein, filtered milk fat, a concentrate of fat globule "membrane" from washed cream, and commercial samples of lactose, riboflavin and ascorbic acid.

## RESULTS

*Effect of processing on the reducing capacity of whole milk.* Chapman and McFarlane (5) have shown that an increase in the temperature of preheating the fluid milk increases the acid ferricyanide reducing substances in spray-dried whole milk. The data presented in table 2 indicate that the reducing capacity of the dry whole milk also is influenced by the temperature of spray drying. The use of higher drying temperatures may, in fact, overshadow the effect of variation in preheating temperature.

TABLE 2

*Effect of preheating and spray-drying on acid ferricyanide reducing substances in dry whole milk*

| Series | Preheat treatment | | Reducing substances as cysteine-HCl per g. of solids | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Temp. | Time | Fresh | Pre-heated | Con-densed | Frozen dried | Spray-dried | |
| | | | | | | | N[a] | H[b] |
| | (°C.) | (*Min.*) | (*mg.*) | (*mg.*) | (*mg.*) | (*mg.*) | (*mg.*) | (*mg.*) |
| 1 | 66 | 30 | ..... | ..... | ..... | ..... | 1.77 | 2.55 |
| 2 | 66 | 30 | 1.00 | 0.96 | 0.96 | ..... | 1.20 | 1.88 |
| 3 | 74 | 30 | 0.96 | 1.00 | 1.08 | 1.11 | 1.24 | 1.58 |
| 4 | 74 | 30 | 1.05 | 1.08 | 1.18 | ..... | 1.90 | ..... |
| 5 | 74 | 30 | 0.86 | 0.86 | ..... | ..... | ..... | ..... |
| 6 | 74 | 30 | ..... | ..... | ..... | 1.22 | 1.63 | ..... |
| 1 | 85 | 20 | ..... | ..... | ..... | ..... | 1.80 | 2.47 |
| 2 | 85 | 20 | 1.00 | 1.14 | 1.09 | ..... | 1.30 | 1.97 |
| 4 | 85 | 30 | 1.05 | 1.21 | ..... | ..... | ..... | ..... |
| 5 | 85 | 30 | 0.86 | 1.02 | ..... | ..... | ..... | ..... |

[a] N = Normal drying temperature.
[b] H = High drying temperature.

As is shown by data in table 2, drying from the frozen state under vacuum is essentially without effect on the reducing capacity of whole milk. This fact has been confirmed in experiments with other samples.

*Contribution of the constituents of milk to the reducing effect.* (a) Caseinate and caseinate-lactose systems—Casein was dispersed in sufficient lime water to produce a sol at pH 6.6 containing 1.0 g. of casein per 16 ml. of sol. Lactose was added to portions of this sol to produce sols with 0, 0.025, 0.05, 0.10, 0.50, 1.0, 2.15 and 4.30 parts of lactose (weighed as $\alpha$-hydrate) per part of casein. The effect of heat treatment at various

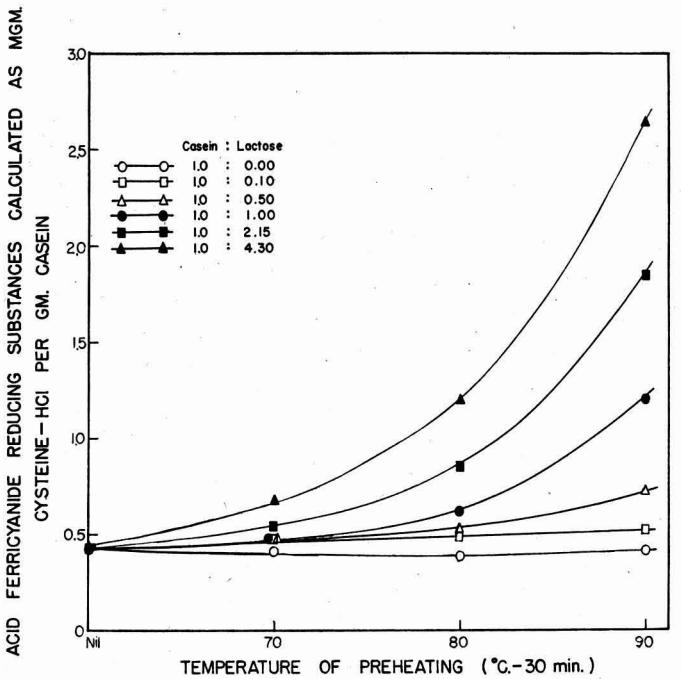


FIG. 5. Effect of heat treatment on acid ferricyanide reducing materials in casein-lactose systems.

temperatures on the acid ferricyanide reducing capacity of these sols is shown in figure 5.

Heat treatment produced no change in the reducing effect of the calcium caseinate sol, but in those sols containing lactose as well as calcium caseinate, there was an increase with increase in temperature, which, at any given temperature, was roughly proportional to the lactose content.

(b) Serum protein and serum-protein-lactose systems—Milk serum protein prepared as described by Jenness and Coulter (9) was equilibrated against phosphate buffer (pH = 6.6, $\mu$ = 0.1) and adjusted to a protein con-

centration of 1.0 g. per 100 ml. Lactose was added to give mixtures containing, respectively, 0, 0.164, 1.632 and 7.06 g. of lactose (weighed as $\alpha$-hydrate) per g. of serum protein. A solution containing 7.06 g. of lactose ($\alpha$-hydrate) per 100 ml. of the buffer but no protein was included for comparison. The effect of heat treatment at various temperatures on the acid ferricyanide reducing capacity of these systems is shown in figure 6.

In contrast to the effect of heat on the calcium caseinate sol, heating of the serum protein sol resulted in an increase in the acid ferricyanide re-

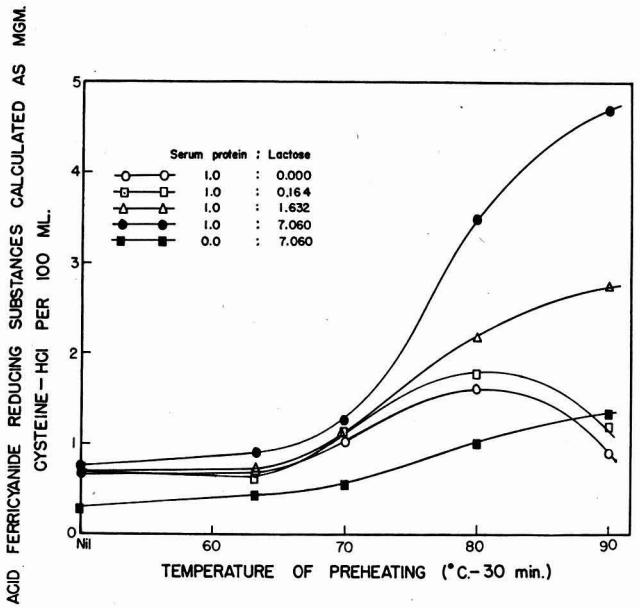


FIG. 6. Effect of heat treatment on acid ferricyanide reducing materials in milk-serum-protein-lactose systems.

ducing effect, but the maximum capacity was not produced by the most drastic heat treatment used. Evidently greater oxidation of reducing groups liberated from the protein occurred on heating at 90° C. Heat treatment of lactose solutions in phosphate likewise produced materials capable of reducing acid ferricyanide. The effect of heat treatment on systems containing both serum protein and lactose was to produce reducing capacities much greater than the sum of those produced from the two constituents separately. These increases were related both to temperature and to the lactose content of the systems.

(c) Systems of calcium phosphocaseinate and other milk constituents dried from the frozen state —As already shown, the drying of whole milk

from the frozen state does not increase its reducing capacity appreciably. The effect of preheating various liquid-simplified systems at temperatures of 74 and of 90° C. for 30 minutes on their reducing capacity after drying from the frozen state is shown in table 3. These systems were prepared as described in a previous paper (9). All of the results have been calculated to the basis of cysteine hydrochloride equivalent per gram of the most complete system (*i.e.*, that containing all of the constituents). Expression of the results in this manner makes immediately apparent the contribution of each constituent to the reducing capacity of the complete system.

The addition of lactose to a caseinate system enhanced both the initial

TABLE 3

*Production of acid ferricyanide reducing substances in simplified systems dried from the frozen state under vacuum*

| System | Constituents[a] | Reducing substances as cysteine-HCl per g. complete system[b] | | |
|---|---|---|---|---|
| | | Heat treatment for 30 min. | | |
| | | None | 74° C. | 90° C. |
| | | *(mg.)* | *(mg.)* | *(mg.)* |
| 1 | Caseinate[c] | 0.05 | 0.05 | ..... |
| 2 | 1 + lactose | 0.14 | 0.20 | 0.30 |
| 3 | 2 + serum protein | 0.20 | 0.28 | 0.35 |
| 4 | 2 + milk fat | ..... | 0.20 | ..... |
| 5 | 4 + f.g.m.[d] | 0.20 | 0.25 | 0.38 |
| 6 | 5 + serum protein | 0.36 | 0.39 | 0.56 |
| 7 | 6 + riboflavin | ..... | 0.33 | ..... |
| 8 | 7 + ascorbic | ..... | 0.50 | ..... |

[a] Ratio of constituents was as follows: 1.00 casein: 2.04 lactose: 0.30 serum protein: 1.52 milk fat: 0.04 f.g.m.[d]: 0.000075 riboflavin: 0.0010 ascorbic acid.
[b] All systems dried from the frozen state under vacuum.
[c] Calcium phospho-caseinate.
[d] Fat globule "membrane".

reduction capacity and the effect of heat, thus confirming the data of figure 5. Serum protein also contributed significantly to reducing capacity and to heat susceptibility, and the materials of the fat globule "membrane" made a small contribution also.

*Production of reducing substances during storage.* The formation of acid-ferricyanide reducing substances during storage of simplified systems dried from the frozen state was studied. Samples of each system were stored under nitrogen over 45 or 60 per cent sulfuric acid at 37 or 50° C. for varying periods of time. Samples of spray dried whole milk and of whole milk dried from the frozen state were included for comparison. The 45 and 60 per cent sulfuric acid solutions furnished vapor pressures at 37° C. comparable to those that had been found to be in equilibrium with dry whole milk containing 5.4 and 2.7 per cent moisture,

TABLE 4

*Reducing capacity of frozen-dried simplified systems and effect of storage thereon*

| System no. | Constituents[a] | Acid ferricyanide reducing substances as cysteine-HCl per g. complete system | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fresh | | | 90 days at 37° C. | | | | 30 days at 50° C. | | | 60 days at 50° C. |
| | | | | | Over 45% $H_2SO_4$ | | Over 60% $H_2SO_4$ | | Over 60% $H_2SO_4$ | | | |
| | | A[b] | B | C | A | B | A | B | A | B | C[c] | B |
| | | *(mg.)* | *(mg.)* | *(mg.)* | *(mg.)* | *(mg.)* | *(mg.)* | *(mg.)* | *(mg.)* | *(mg.)* | *(mg.)* | *(mg.)* |
| 1 | Caseinate | ..... | ..... | 0.05 | ..... | ..... | ..... | ..... | ..... | ..... | 0.08 | ..... |
| 2 | 1 + lactose | 0.15 | 0.17 | 0.20 | 4.95 | 4.90 | 0.41 | 0.43 | 1.11 | 0.63 | 1.90 | 1.46 |
| 3 | 2 + serum prot. | 0.22 | 0.28 | 0.28 | 6.47 | 6.03 | 0.72 | 0.67 | 1.90 | 0.99 | 2.72 | 3.40 |
| 4 | 2 + milk fat | 0.20 | 0.21 | 0.20 | 5.58 | 4.98 | 0.60 | 0.55 | 1.10 | 0.91 | 1.45 | 2.13 |
| 5 | 4 + f.g.m.[d] | 0.19 | 0.25 | 0.25 | 5.50 | 4.84 | 0.77 | 0.56 | 1.60 | 0.84 | 1.77 | 2.07 |
| 6 | 5 + serum prot. | 0.32 | 0.38 | 0.39 | 7.46 | 5.88 | 0.97 | 0.70 | 2.41 | 1.24 | 1.72 | 3.09 |
| 7 | 6 + riboflavin | 0.22 | 0.35 | 0.33 | 7.85 | 5.86 | 0.92 | 0.72 | 2.25 | 1.26 | 1.99 | 3.06 |
| 8 | 7 + ascorbic | 0.49 | 0.53 | 0.50 | 8.16 | 5.97 | 1.08 | 0.84 | 2.05 | 1.62 | 2.46 | 2.99 |
| | *Whole milk:* | | | | | | | | | | | |
| 9 | Frozen dried | | 1.22 | | 10.28 | | 2.05 | | 3.00 | | ..... | ..... |
| 10 | Spray dried | | 1.63 | | 8.81 | | 2.33 | | 3.07 | | ..... | ..... |

[a] Ratio of constituents was as follows: 1.00 casein: 2.15 lactose hydrate: 0.30 serum protein: 1.52 milk fat: 0.04 f.g.m.[d]: 0.000075 riboflavin: 0.0010 ascorbic acid.

[b] Letters designate replicate series.

[c] Stored 34 days.

[d] Fat globule "membrane".

respectively. The quantities of simplified systems prepared, particularly those containing serum protein, were insufficient to permit satisfactory moisture determinations by the toluene distillation method.

Here again the results have been calculated to the basis of cysteine hydrochloride equivalent per gram of complete system. The data, recorded in table 4 show that even the most complete system employed failed to exhibit a reducing capacity of over half of that of dry whole milk. There was only a slight increase in reducing capacity of the caseinate system during storage. Interaction of caseinate and lactose was responsible for the major portion of the increase produced in storage. Serum protein also contributed materially to the original- and storage-produced reducing

TABLE 5

*Non-protein reducing capacity of frozen-dried simplified systems and effect of storage thereon*

| System no. | Constituents | Acid ferricyanide reducing substances as cysteine-HCl per g. complete system | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Fresh | | 90 days at 37° C. | | | | 30 days at 50° C. | |
| | | | | Over 45% $H_2SO_4$ | | Over 60% $H_2SO_4$ | | Over 60% $H_2SO_4$ | |
| | | A | B | A | B | A | B | A | B |
| | | *(mg.)* | *(mg.)* | *(mg.)* | *(mg.)* | *(mg.)* | *(mg.)* | *(mg.)* | *(mg.)* |
| 2 | Caseinate + lactose | 0.03 | 0.05 | 0.65 | 0.45 | 0.12 | 0.14 | 0.13 | 0.10 |
| 3 | 2 + serum prot. | 0.05 | 0.09 | 1.00 | 0.62 | 0.22 | 0.17 | 0.20 | 0.22 |
| 4 | 2 + milk fat | 0.06 | 0.04 | 0.68 | 0.37 | 0.27 | 0.15 | 0.08 | 0.16 |
| 5 | 4 + f.g.m. | 0.07 | 0.09 | 0.53 | 0.32 | 0.12 | 0.15 | 0.15 | 0.21 |
| 6 | 5 + serum prot. | 0.08 | 0.08 | 0.65 | 0.41 | 0.20 | 0.13 | 0.35 | 0.16 |
| 7 | 6 + riboflavin | 0.08 | 0.06 | 0.85 | 0.41 | 0.27 | 0.16 | 0.35 | 0.22 |
| 8 | 7 + ascorbic | 0.35 | 0.21 | 0.94 | 0.57 | 0.35 | 0.19 | 0.50 | 0.33 |
| | *Whole milk:* | | | | | | | | |
| 9 | Frozen-dried | 0.71 | | 2.41 | | 0.77 | | 0.99 | |
| 10 | Spray-dried | 0.68 | | 1.70 | | 0.77 | | 1.04 | |

capacities. The effect of the fat globule "membrane" materials was small and variable. Milk fat and riboflavin were inert but, as expected, ascorbic acid definitely enhanced the reducing capacity.

The data in table 5 indicate that ascorbic acid is the major non-protein reductant of acid ferricyanide in the fresh systems, but that non-protein reducing materials are produced upon storage by reactions involving the proteins or lactose or both. As was the case with total reducing capacity, the most complete simplified system failed to account for all of the non-protein reducing capacity of dry whole milk.

## DISCUSSION

The modified ferricyanide method in which the pH is raised to 6.6 and the temperature lowered to 50° C. appears to yield as satisfactory re-

sults as that originally described by Chapman and McFarlane. It has the advantage of eliminating formation of a blue precipitate during the heating.

Ascorbic acid has been found to reduce ferricyanide stoichiometrically under the conditions adopted for the reaction. The amount of ascorbic acid present in milk, however, accounts for only a fraction of the ferricyanide-reducing capacity. Thus, for a milk containing 20 mg. of reduced ascorbic acid per l. (125 g. of solids), it may be calculated from the standardization curves that ascorbic acid would account for a reducing capacity equivalent to 0.36 mg. cysteine hydrochloride per g. of solids out of a total of about 1.00 mg. per g. Actually the effect of addition of ascorbic acid in the amount of 25 mg. per liter to artificial systems was somewhat less than this, amounting to the equivalent of 0.27, 0.18 and 0.17 mg. cysteine hydrochloride per g. in the three series reported in table 4. Obviously, considerable differences in the acid ferricyanide reducing capacity of milk may result from variation in the degree of oxidation of its ascorbic acid content.

Such relatively simple sulfhydryl compounds as cysteine and glutathione are also effective reductants of ferricyanide under the conditions used. According to Brand and Kassell (4) the cysteine content of $\beta$-lactoglobulin is about 1.10 per cent (analyzed after acid hydrolysis). If this figure be assumed to apply to the entire 0.70 per cent serum protein of milk, and if the sulfhydryl groups of milk proteins were as reactive as those of cysteine or glutathione, the protein sulfhydryls of milk would furnish a reducing capacity equivalent to about 0.80 mg. cysteine hydrochloride per g. of solids. Actually, the fact that the contribution of milk serum protein falls far short of this value (see table 4) constitutes evidence that the sulfhydryls of protein are much less active than those of the simpler compounds.

The failure of the most complete simplified system prepared to exhibit more than one-half the reducing capacity of fresh or frozen-dried whole milk could conceivably be due to absence of some milk reducing system from the simplified preparation. On the other hand, the reducing capacity of one or more of the constituents of the simplified system might possibly have been altered in isolation and purification. Such an effect would be most probable with the serum protein. A third possibility is that the environment of the reducing materials in the simplified systems is different enough from that in milk to account for the difference. The results reported in this paper give no clue to the reason for the discrepancy.

In spite of the failure quantitatively to duplicate the reducing capacity of fresh whole milk, the simplified systems do exhibit increases in reducing capacity upon heat treatment and storage which are quite comparable to those observed with milk itself. Furthermore, they indicate that these in-

creases are due to materials formed in part by reactions of the proteins with lactose and in part by reactions involving lactose in the presence of buffer salts.

The method is being applied to further study of factors influencing the changes occurring in dry whole milk during storage.

## SUMMARY AND CONCLUSIONS

A modification of Chapman and McFarlane's ferricyanide procedure for evaluating the reducing capacity of milk is presented. This modification, which involves raising the pH to 6.6 and lowering the temperature to 50° C., proved somewhat more satisfactory, particularly with milk powders of high reducing capacity, than the original method. The method has been calibrated in terms both of ferricyanide reduced and of cysteine or ascorbic acid oxidized.

The capacity of milk to reduce ferricyanide is increased both by heat treatment and by spray drying. Study of simplified systems of milk constituents has shown that some of the reducing substances produced by heat treatment of milk and aging of dry milk are formed from lactose and from protein-lactose interactions.

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# THE INFLUENCE OF *MYCOTORULA LIPOLYTICA* LIPASE UPON THE RIPENING OF BLUE CHEESE MADE FROM PASTEURIZED HOMOGENIZED MILK[1]

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The present trend in the dairy industry is to manufacture cheese from pasteurized milk, both for public health reasons and to aid in the control of microbial defects. When blue cheese is made from pasteurized milk the product does not develop a full and typical flavor during ripening (6, 10); this has been attributed primarily to the inactivation of milk lipase by pasteurization (10), resulting in less hydrolysis of the butterfat of the cheese. The solution of this problem seems to lie in the substitution of a suitable lipolytic enzyme for the milk lipase inactivated by pasteurization. The present study was undertaken to explore the possibility of substituting the cell-free lipase produced by *Mycotorula lipolytica* (15, 16) for normal milk lipase in the manufacture of blue cheese from pasteurized homogenized milk.

## HISTORICAL

Methods for the manufacture of blue cheese from raw cows' milk have been described by different workers (6, 12, 18). Lane and Hammer (9) modified the procedure formerly used by homogenizing the raw milk. This modification resulted in faster ripening of the cheese as well as in more luxurious mold growth, as compared with similar cheese made from nonhomogenized milk. Later the same workers (10) reported that blue cheese made from pasteurized homogenized milk was a more satisfactory product than that made from raw, nonhomogenized milk, but less satisfactory than if raw homogenized milk was used. They also observed that milk lipase definitely aided in the ripening of blue cheese. Fabricius and Nielsen (5) were able to produce a satisfactory blue cheese from raw, nonhomogenized milk by using a combination of heat and vacuum treatment of milk, namely 165–175° F. and 19 inches of vacuum. This treatment destroyed most of the undesirable microorganisms present in the raw milk without inactivating the milk lipase.

Irvine (8) added a commercial lipase preparation, later reported as steapsin (13), at the rate of 0.5 and 1.0 g. per 100 lb. of raw milk; the addition of the enzyme preparation resulted in accelerated fat hydrolysis and quicker ripening of the cheese as compared with the control, but a bitter flavor resulted in the cheese. Similar results were obtained by Coul-

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ter and Combs (3), who also used steapsin to hasten the ripening of blue cheese made from raw nonhomogenized milk. Thibodeau and Macy (17) added the enzymes of *Penicillium roqueforti* in the form of mycelium at the time blue cheese made from raw milk was hooped. The addition of 6 g. of mycelium to 5 lb. of cheese reduced the curing period from 10 to 5 months total time, as compared with the control without added mycelium. Parmelee (14) added pure cultures of *Alcaligenes lipolyticus, Achromobacter lipolyticum, Pseudomonas fragi* and *Mycotorula lipolytica* separately to lots of pasteurized homogenized milk made into blue cheese, and found that of these microorganisms only certain strains of *M. lipolytica* improved the flavor score and increased significantly the total volatile acidity of the cheese. This investigator also added to pasteurized homogenized milk special cultures of *P. roqueforti* grown on a modification of Czapek's medium containing 10 per cent butterfat and obtained cheese which were much superior to those made without the added special culture.

## METHOD

Regular pasteurized homogenized milk of 3.5–3.8 per cent butterfat content was used in all experiments in quantities of 105 to 110 lb. per vat or lot. Three or four vats were used at one time, comprising a series, and conditions were kept as uniform as possible throughout the manufacture of cheese in each series. The vat contents were kept at 90° F. from the time of adding the culture until the curd was hooped. One per cent starter was used, and rennet was added at the rate of 90 ml. per 1,000 lb. of milk after 30 minutes holding time in series 1 to 4, and after 60 minutes holding time in series 5 to 7. Calcium chloride was added at the rate of 0.015 per cent (7 g. per 100 lb. of milk) to the milk in series 3 to 7 prior to setting. At the same time a previously standardized cell-free lipase preparation from *M. lipolytica* (16) was added in definite quantities to all but the control lot of milk in each series. The curd was cut into 0.5 inch cubes 70 minutes after setting and held for 2 hours with some stirring every 30 minutes, after which time the whey was drained. One per cent salt and 0.01 per cent mold powder were added to the curd at the time of hooping. The cheese was dry-salted daily for 4 days, using a total of 5 lb. of salt per 100 lb. of curd. Next the cheese was skewered and placed in the ripening room at approximately 10° C. and a relative humidity of approximately 90 per cent, where it remained for 12 weeks.

The cheese were examined and scored for positive flavor, defects, and visual mold growth after ripening periods of 4 and 12 weeks. A score of 10 was considered perfect in each of the three items under consideration. The total volatile acidity of the cheese was determined by the method of Lane and Hammer (10) at the ages of 4 and 12 weeks. Determinations of moisture, fat and total chlorides in the cheese at 4 weeks showed only

TABLE 1

*Preliminary trials on the influence of the addition of various amounts of M. lipolytica lipase upon the liberation of volatile free fatty acids, flavor and mold score of ripening blue cheese*

| Lot no. | Amount of lipase added[a] | Vol. acidity in ml. 0.1 *N* acid per 200 g. of cheese | | Score | | | | | | Remarks on flavor at 12 weeks |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Flavor | | | | Mold | | |
| | | | | Positive | | Negative | | | | |
| | | 4 weeks | 12 weeks | 4 weeks | 12 weeks | 4 weeks | 12 weeks | 4 weeks | 12 weeks | |
| | | | | | Series 1 | | | | | |
| 11 | None | 6.0 | 18 | .....[b] | ..... | ..... | ..... | ..... | ..... | Lacking, slightly unclean |
| 12 | 100 | 6.6 | 18 | ..... | ..... | ..... | ..... | ..... | ..... | Lacking, slightly unclean |
| 13 | 200 | 7.0 | 16 | ..... | ..... | ..... | ..... | ..... | ..... | Lacking, sl. unclean, bitter |
| | | | | | Series 2 | | | | | |
| 21 | None | 5.4 | 13 | ..... | ..... | ..... | ..... | ..... | ..... | Lacking, sl. unclean |
| 22 | 300 | 9.6 | 22 | ..... | ..... | ..... | ..... | ..... | ..... | Sl. soapy, sl. unclean |
| 23 | 800 | 18.0 | 65 | ..... | ..... | ..... | ..... | ..... | ..... | Sl. sharp, soapy |
| | | | | | Series 3 | | | | | |
| 31 | None | 7.0 | 13 | 3.0 | 4.0 | 4.0 | 3.5 | 3.5 | 4.0 | Lacking, sour |
| 32 | 660 | 26.0 | 50 | 7.0 | 7.5 | 6.5 | 7.0 | 4.0 | 7.0 | Fair, sl. sharp, sl. soapy, sl. sour |
| 33 | 1300 | 39.0 | 85 | 6.5 | 6.0 | 5.5 | 3.0 | 3.5 | 5.0 | Excessively sharp and soapy |
| | | | | | Series 4 | | | | | |
| 41 | None | 9.0 | 18 | 3.5 | 4.0 | 6.0 | 3.5 | 7.5 | 7.5 | Lacking, sl. nutty, sl. fermented |
| 42 | 300 | 15.0 | 38 | 6.5 | 5.0 | 6.0 | 4.0 | 3.5 | 7.0 | Fair, sl. nutty, sl. unclean |
| 43 | 600 | 21.0 | 60 | 8.0 | 7.5 | 8.0 | 7.0 | 5.5 | 4.5 | Sl. sharp, sl. soapy |
| 44 | 900 | 26.0 | 72 | 7.0 | 5.5 | 5.5 | 3.5 | 3.5 | 6.0 | Excessively sharp and soapy |

[a] Calculated as total lipase activity (ml. of preparation × lipase activity per ml.), the activity being expressed in acid degrees, which are defined as ml. of *N* NaOH required to neutralize the free fatty acids in 100 g. of fat (1).

[b] No numerical score given.

TABLE 2

*Further trials on the influence of the addition of various amounts of M. lipolytica lipase upon the liberation of volatile free fatty acids, flavor and mold score of ripening blue cheese*

| Lot no. | Amount of lipase added[a] | Vol. acidity in ml. 0.1 *N* acid per 200 g. of cheese | | Score | | | | | | Remarks on flavor at 12 weeks |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Flavor | | | | Mold | | |
| | | | | Positive | | Negative | | | | |
| | | 4 weeks | 12 weeks | 4 weeks | 12 weeks | 4 weeks | 12 weeks | 4 weeks | 12 weeks | |
| | | | | | Series 5 | | | | | |
| 51 | None | 6.5 | 20.0 | 4.0 | 4.0 | 3.0 | 4.0 | 6.0 | 6.5 | Lacking, musty, unclean |
| 52 | 250 | 10.5 | 27.4 | 5.0 | 4.5 | 4.5 | 5.5 | 6.0 | 2.5 | Lacking, musty, sl. unclean |
| 53 | 375 | 10.5 | 33.7 | 5.5 | 6.0 | 6.0 | 5.0 | 5.5 | 5.0 | Sl. lacking, sour, sl. unclean |
| 54 | 500 | 12.0 | 37.4 | 6.0 | 7.0 | 6.0 | 7.0 | 5.0 | 6.5 | Fair, sl. sour, sl. unclean |
| | | | | | Series 6 | | | | | |
| 61 | None | 8.0 | 21.6 | 3.0 | 4.0 | 1.0 | 3.5 | 6.0 | 4.5 | Lacking, bitter, musty, sl. sour |
| 62 | 250 | 11.0 | 31.2 | 6.0 | 4.5 | 6.0 | 4.5 | 5.0 | 4.5 | Lacking, bitter, musty, sour |
| 63 | 375 | 11.0 | 34.6 | 5.5 | 6.0 | 5.0 | 7.0 | 6.0 | 5.0 | Sl. lacking, sl. sour, sl. unclean |
| 64 | 500 | 13.0 | 35.4 | 6.5 | 6.5 | 6.0 | 6.0 | 5.0 | 7.0 | Fair, sour, nutty |
| | | | | | Series 7 | | | | | |
| 71 | None | 7.0 | 19.5 | 4.0 | 4.5 | 3.5 | 5.0 | 6.0 | 6.0 | Lacking, musty, sl. sour, fermented |
| 72 | 250 | 9.0 | 29.3 | 5.0 | 6.5 | 5.0 | 7.5 | 7.5 | 6.5 | Lacking, sl. sour, sl. unnatural |
| 73 | 375 | 10.5 | 33.0 | 6.0 | 6.5 | 6.5 | 4.5 | 5.5 | 7.5 | Sl. lacking, musty, unclean |
| 74 | 500 | 13.0 | 38.2 | 7.0 | 7.5 | 6.0 | 6.5 | 5.0 | 6.5 | Fair, sharp, unnatural, sl. sour |

[a] Calculated as total lipase activity (ml. of preparation × acid degree value per ml.) added to 105 lb. of milk.

slight variations within each series; these differences were not considered significant in the enzyme study under consideration, and therefore the data are not presented in this paper.

## RESULTS

Data showing the influence of the addition of various amounts of *M. lipolytica* lipase upon the volatile acidity, flavor and mold growth of the cheese in series 1 to 4 are presented in table 1. The trials were of a preliminary nature and served to indicate the amount of enzyme required for the production of a blue cheese in which a satisfactory level of fat hydrolysis occurred. The control cheese (lots 11, 21, 31, 41) were lowest in total volatile acidity in their respective series and were lacking completely in the desired ketone flavor characteristic of properly ripened blue cheese. Additions of *M. lipolytica* lipase to the milk resulted in increases in the total volatile acidity of the cheese in proportion to the amount of lipase added. Cheese with total volatile acidity values of 50 and above at 12 weeks were criticized for being soapy and sharp, both characteristics being undesirable (lots 23, 32, 33, 43, 44). Lots 32 and 43 were most satisfactory from both body and flavor standpoint, although they also were criticized for being slightly soapy and slightly sharp.

Table 2 shows the results of replicate series 5, 6 and 7 made within a 5-day period after the complete data of the first four series had been collected. Again the total volatile acidity values of the controls (lots 51, 61, 71) were the lowest in each respective series, with the values increasing in the order of increasing enzyme concentration of the cheese. A close correlation existed between the total volatile acidity values of the cheese in the three series and the concentration of enzyme used. The flavor score, and to a certain extent also the defect score, showed good correlation with the total volatile acidity values, highest scores being given to lots 54, 64 and 74 which showed total volatile acidity values at 12 weeks of 37.4, 35.4 and 38.2, respectively. None of the cheese in these series was criticized for soapiness or excessive sharpness, although other defects were encountered; however, these could not be attributed to the enzyme added. This was also true in the first four series (table 1).

There was no indication in the cheese of any one of the seven series that mold growth was affected by the different amounts of total volatile acidity present at any time in the individual lot of cheese. No correlation could be established between mold score and flavor score of any one cheese. Although the mold scores of the different cheese varied from 4 to 7.5, all of the cheese showed sufficient mold growth to permit flavor development if other conditions were satisfactory.

## DISCUSSION

The addition of *M. lipolytica* lipase to pasteurized milk which then was made into blue cheese brought about the desired hydrolysis of the fat.

The acidity of the cheese and the temperature at which the cheese was ripened both were favorable for the action of the lipase, as had been anticipated from previous study of this enzyme system (16). A good relationship existed between the amount of enzyme added and the values for total free volatile fatty acid obtained at 4 and 12 weeks of ripening of the cheese. The cheese containing the added lipase had more organoleptically detectable free fatty acids, as well as ketone flavor, and a waxier body than the control cheese without added lipase. These observations suggest that the lipase added was of considerable value in aiding in the proper ripening of blue cheese. According to Lane and Hammer (10), a satisfactory ripened blue cheese was not obtained until after 16 weeks holding time, when pasteurized, homogenized milk was used, while with raw homogenized milk a satisfactory ripened cheese was obtained in 12 weeks. Thus the presence of lipase, either milk lipase or added lipase such as used in this study, brings about early hydrolysis of the fat and thus enables the mold to utilize the free fatty acids and to change certain ones into flavor-producing ketones (7).

The sharp, soapy taste in a number of cheese was correlated with free fatty acid values of 50 and higher in the cheese after ripening for 12 weeks. Cheese with most desirable flavor at this age showed values between 30 and 50. Other workers have made observations which support this conclusion (10, 14). The flavor of the cheese containing the added lipase, while characteristic of blue cheese, did not duplicate exactly the flavor of the product made from homogenized raw milk. However, most of those who sampled the cheese made from pasteurized homogenized milk containing the added lipase accepted the product as satisfactory cheese with a high level of good flavor development. Under no circumstances was a bitter or other objectionable flavor definitely attributable to the addition of the microbial lipase. Less breakdown of the body to a desirable level was observed in the controls than in the cheese made with added lipase. Since *M. lipolytica* is both lipolytic and proteolytic, it is possible that the cell-free lipase preparation also carried some proteolytic enzymes which were beneficial to the breakdown of the protein in the cheese. No data were collected on this phase of cheese ripening, although a study of this point would be desirable.

The repeatedly observed close relationship between total activity of lipolytic enzyme preparation added to the milk and extent of fat degradation in the resulting cheese permitted the addition of predetermined amounts of enzyme which would result in the desired level of total volatile acidity in the cheese after the ripening period of 12 weeks employed in this study.

The data indicate that cell-free lipase obtained from cultures of *M. lipolytica* could be used to advantage in the manufacture of blue cheese

made from pasteurized homogenized milk, and possibly also in other varieties of cheese in which hydrolysis of fat is essential for the proper ripening of the cheese.

## SUMMARY AND CONCLUSIONS

1. Seven series of blue cheese were made from pasteurized homogenized milk with and without the addition of a cell-free lipase preparation obtained from *Mycotorula lipolytica.*

2. Examinations of the cheese at 12 weeks for flavor and other desirable characteristics showed the cheese ripened with the aid of the cell-free lipase preparation consistently was more satisfactory than the corresponding control containing no added lipase.

3. Increases in the concentration of lipase in the cheese resulted in increases in total volatile acidity values of the cheese and also of the intensity of the flavor typical of blue cheese. Cheese with enzyme concentrations high enough to show total volatile acidity values of from 30 to 50 after ripening for 12 weeks were most satisfactory in flavor. Cheese with total volatile acidity values above 50 were criticized as being sharp and soapy in every case.

4. The results of this study indicate that the cell-free lipase prepared from cultures of *M. lipolytica* can be used advantageously in the ripening of blue cheese made from pasteurized homogenized milk.

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# MOISTURE STUDIES IN DRY PRODUCTS OF MILK. II. ESTIMATING WATER OF CRYSTALLIZATION OF ALPHA-LACTOSE IN DRY WHEY SOLIDS

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In the previous paper on the kinetics of moisture desorption of crystalline *alpha*-lactose hydrate (1), a possible method was suggested for estimating the water of crystallization of lactose in dry products of milk. In the present communication experimental evidence in support of the method, together with results obtained on dry whey solids, is presented. Dry whey solids have been selected for study, because samples with the lactose largely in the form of the crystalline hydrate are readily available.

The lactose in nonfat dry milk solids and dry whey solids manufactured by the ordinary spray and roller processes has been reported to be amorphous (5, 7). In recent years, however, various processes have been developed for inducing crystallization of lactose as the *beta*-anhydride or as the *alpha*-hydrate in dry whey solids (5).

Sharp *et al.* (6) have found that the state of lactose in dry products of milk has a great influence on the determination of moisture by the toluene distillation method. For products containing crystalline lactose hydrate, a longer distillation is necessary than for similar products in which the lactose is in the amorphous state. Presumably, the loss of moisture at the later stage is due to the dehydration of crystalline *alpha*-lactose hydrate. In a previous study on crystalline *alpha*-lactose hydrate in boiling toluene (1), this laboratory observed that the rate of dehydration follows the first order kinetics expression,

$$k = \frac{2.303}{t} \log \frac{a}{(a-x)}$$

where $k$ is the rate constant, $a$ the initial moisture content, and $x$ the amount of moisture removed in time $t$. Therefore, it appears possible to estimate the water of crystallization of *alpha*-lactose and consequently crystalline lactose hydrate by taking advantage of this difference in the rates of moisture removal and of the unimolecular character of the dehydration of crystalline lactose hydrate.

## EXPERIMENTAL PROCEDURE

*Moisture desorption method.* The apparatus used was exactly the same as that employed previously in the study on crystalline *alpha*-lactose hydrate (1). Fifty grams of sample were weighed into the 300-ml. Erlenmeyer flask and quickly covered with 100 ml. of moisture-free toluene. After at-

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taching the flask containing the sample to the apparatus, toluene was added through the top of the condenser to fill the moisture trap. Stirring then was applied to keep the mixture well agitated. The rate of distillation was adjusted to give more than two drops per second (1). At 5- or 10-minute intervals after the first appearance of moisture in the trap, the volume of water collected was read and multiplied by two to convert to per cent of

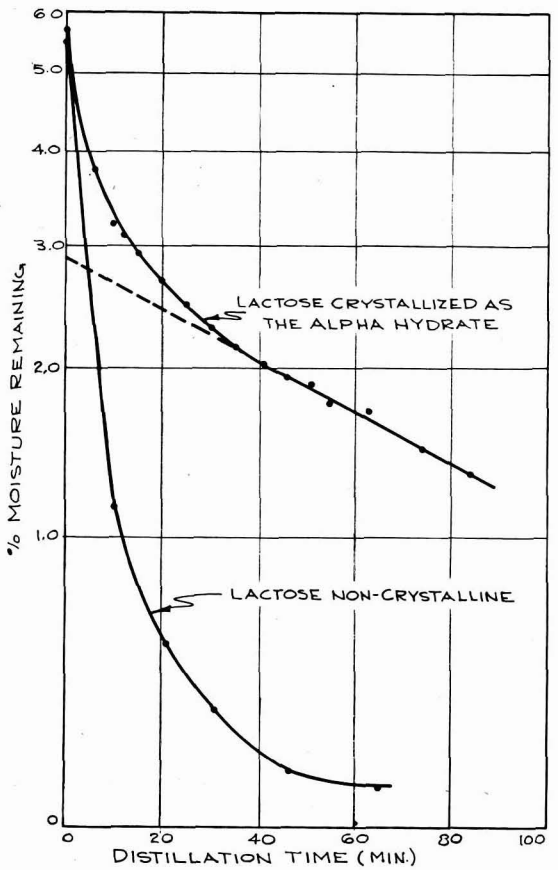


FIG. 1. Typical moisture desorption curves of two different types of dry whey solids.

water desorbed. The total moisture was determined by the Karl Fischer method (2). Less accurately, it may be estimated by continued distillation until a sufficiently constant value is obtained; this generally requires about 3 hours for the type of dry whey solids studied. The logarithm of the per cent moisture remaining in the sample, *i.e.*, log $(a-x)$, at each time interval was plotted against the distillation time in minutes. The top curve (fig. 1) has two portions. The initial steep portion represents the dehydration of

crystalline *alpha*-lactose hydrate together with the protein hydrates and surface moisture. The second portion is linear and represents the dehydration of crystalline *alpha*-lactose hydrate. Consequently, by extrapolating the straight-line portion to zero time, the initial percentage of water from crystalline *alpha*-lactose hydrate may be obtained by taking the antilogarithm of the vertical intercept. If desired, the percentage of crystalline *alpha*-lactose hydrate present in each sample can be obtained by dividing the determined per cent of water of crystallization by 0.050.

*Indirect method.* The indirect method referred to in table 1 is a combination of two determinations: (a) total moisture by the Karl Fischer pro-

TABLE 1

*Water of crystallization of alpha-lactose in some dry whey solids by two methods*

| Sample no. | Indirect method | | | Desorption method |
|---|---|---|---|---|
| | % Total $H_2O$ (Karl Fischer) | % Free $H_2O$ (vac. oven) | % $H_2O$ crystallization | |
| | | | | *(%)* |
| 1 | 5.68 | 2.73 | 2.95 | 2.89 |
| 2 | 5.31 | 2.55 | 2.76 | 2.70 |
| 3 | 5.44 | 2.82 | 2.62 | 2.73 |
| 4 | 5.27 | 2.48 | 2.79 | 2.89 |
| 5 | 5.79 | 3.08 | 2.71 | 2.52 |
| 6 | 4.47 | 1.89 | 2.58 | 2.60 |
| 7 | 5.43 | 2.68 | 2.75 | 2.67 |
| 8 | 4.91 | 2.00 | 2.91 | ..... |
| 9 | 3.88 | 1.23 | 2.65 | 2.69 |
| 10 | 5.76 | 3.10 | 2.66 | 2.95 |
| 11 | 4.21 | 1.71 | 2.50 | 2.62 |
| 12 | 3.81 | 1.38 | 2.43 | 2.40 |
| 13 | 4.33 | 1.84 | 2.49 | 2.51 |
| 14 | 3.70 | 1.30 | 2.40 | 2.45 |
| 15 | 3.67 | 0.87 | 2.80 | 2.88 |
| 16 | 4.93 | 2.13 | 2.80 | 2.94 |

cedure of Fosnot and Haman (2) using visual end-point estimation and (b) "free" moisture by dehydration of a 4-g. sample in a Cenco-DeKhotinsky vacuum oven at 65° C. and 2–3 mm. mercury pressure for 5 hours (4). This method again is based upon the fact that lactose hydrate dehydrates at an extremely slow rate under the conditions used in the determination of "free" water. Thus, in two experiments with crystalline lactose hydrate of particle sizes less than 149 $\mu$, only 0.05 and 0.07 per cent of moisture were removed in 5 hours. On the other hand, dry casein containing approximately 8 per cent moisture appeared to be completely dehydrated. The difference between the Karl Fischer result and that obtained in the low temperature vacuum oven determination was inferred to be water of crystallization of *alpha*-lactose.

*Samples.* All samples of dry whey solids used in this study were selected from samples currently sent to this laboratory for analysis. The particle

sizes of these products generally are within the range of about 54 to 210 $\mu$. Crystalline *alpha*-lactose hydrate was Baker's C.P. powder containing the theoretical 5.0 per cent water of crystallization as determined by the Karl Fischer method (2). The particle sizes were under 149 $\mu$. Dry casein was of technical grade obtained from J. T. Baker Chemical Company.

## RESULTS

Figure 1 shows two typical moisture desorption curves for two different types of dry whey solids. For the top curve the lactose in the product is

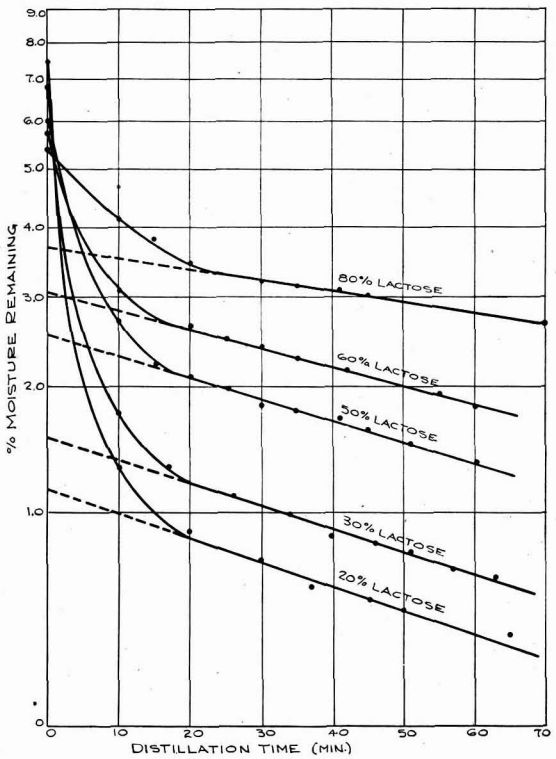


FIG. 2. Dehydration curves of mixtures of *alpha*-lactose hydrate and casein.

present largely in the form of the crystalline *alpha* hydrate, as indicated by the seeding test used by Troy and Sharp (7). This curve illustrates the initial rapid loss of moisture and the slower constant desorption after the first 20–30 minutes. For the lower curve the lactose is in the glass or amorphous state, as shown by a negative seeding test. This type of dry whey solids forms a single hard mass in boiling toluene and, in spite of the resultant reduction of surface area, shows a rapid rate of dehydration.

Since protein and lactose are the two major constituents in most dry products of milk, the method was applied to mixtures containing different proportions of dry casein and lactose hydrate to see how well the latter can be recovered. Results are plotted in figure 2. The casein used for the first two trials contained approximately 7 per cent moisture and was less than 149 $\mu$ in particle size. The remaining trials were conducted with dry casein of slightly higher moisture content and of particle size less than 210 $\mu$.

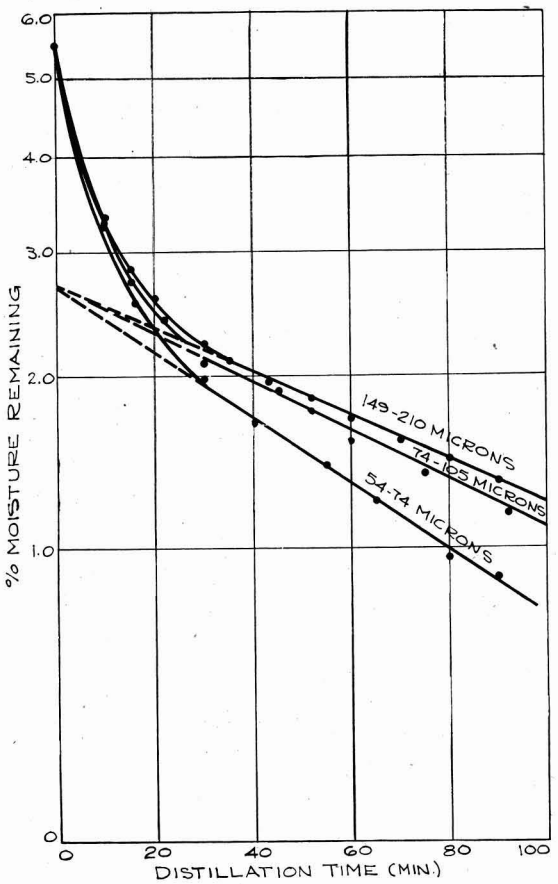


FIG. 3. Effect of particle size on the determination of *alpha*-lactose hydrate in dry whey solids.

A comparison of results by the desorption method with those by the indirect method on samples of dry whey solids which gave positive seeding tests for crystalline *alpha*-lactose hydrate is presented in table 1. Under conditions employed in the vacuum oven test for "free" moisture, weight loss after 5 hours of heating was constant.

In order to determine whether particle size has any effect on the final result, a sample of dry whey solids was fractionated into different particle sizes by means of standard sieves. Results are plotted in figure 3. The straight lines used for extrapolation were calculated by the method of least squares.

## DISCUSSION

The difference in moisture desorption behavior of the two types of dry whey solids is quite evident from figure 1. It can be seen that the presence of crystalline *alpha*-lactose hydrate gives rise to a slower rate of desorption, which is unimolecular at the later stage. Since the composition of the two types of dry whey solids is approximately the same, it is unlikely that this difference in the rate of desorption could have arisen from any other sources.

The results of the experiments using crystalline *alpha*-lactose hydrate and dry casein, as shown in figure 2, indicate that at least in simple mixtures of the two materials, water of crystallization of lactose can be quantitatively differentiated from water adsorbed by casein. Moreover, the similarity of curve 1 shown in figure 1 to those in figure 2 tends to support the previous interpretation of each portion of the curve.

The apparent agreement between results obtained by the moisture desorption method and the indirect method as shown in table 1, in all probability, is not accidental. Admittedly, both methods are based upon the slowness in the dehydration of crystalline *alpha*-hydrate as compared with other moisture adsorbing constituents. Yet the two methods differ entirely in other respects. Whereas one method determines water of crystallization of lactose hydrate from the difference between total and "free" moisture, the other depends upon the unimolecular character of the dehydration of crystalline lactose hydrate in boiling toluene. Agreement between the two series of results must be considered good in view of the fact that the moisture determinations by even the best available methods usually involve deviations of the magnitude of 0.1–0.2 per cent.

Referring to figure 3, particle size within the range studied does not seem to have any influence on the extrapolated value in the desorption method aside from changing the rate of dehydration. Ideally, a sample should be homogeneous with respect to particle size. Practically, it has been found that for particle size occurring normally in dry whey solids of the type studied, the linear relationship for the dehydration of crystalline *alpha*-lactose hydrate still is obeyed.

From the above evidence it appears that both the moisture desorption method and the indirect method can be used for estimating water of crystallization of lactose and consequently of *alpha*-lactose hydrate itself in certain dry whey solids. Presumably, the methods can be applied to other dry products of milk containing crystalline lactose hydrate. It must be

pointed out that the desorption method depends on the continued presence of crystalline *alpha*-lactose hydrate after complete removal of all other forms of moisture. For this reason the method may not be as accurate for products containing small quantities of crystalline *alpha*-lactose hydrate as for the dry whey solids studied.

SUMMARY

A moisture desorption method has been developed for estimating the water of crystallization of *alpha*-lactose and indirectly the crystalline *alpha* hydrate itself in certain types of dry whey solids. It is based upon the difference in the rates of dehydration of crystalline *alpha*-lactose hydrate and other moisture adsorbing constituents and also upon the unimolecular dehydration of the hydrate itself.

Good recovery was obtained using mixtures of known composition of crystalline *alpha*-lactose hydrate and casein.

Results obtained by this method were in close agreement with those by an indirect method in which the difference between total moisture as determined by the Karl Fischer method and "free" moisture as determined by an oven procedure was considered to be water of crystallization of *alpha*-lactose.

Aside from an effect on the rate of dehydration, particle size, within the range of 54 to 210 $\mu$, was found to have no influence on the results obtained by the moisture desorption method.

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