A STUDY OF OXIDIZED FLAVOR IN RAW MILK IN RELATION TO CERTAIN MILK CONSTITUENTS

by

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INTRODUCTION

Milk is proclaimed as the greatest of human foods, yet its use is diminished by the frequent occurrence of objectionable off-flavors. Although it is not as common as some of the other off flavors, oxidized flavor is one of the most objectionable flavors found in milk. It's sporadic occurrence offers a serious problem to milk companies because it is so difficult to control. During recent years, in face of the greater sanitary precautions, and the increased use of pasteurization, oxidized flavored milk has become increasingly prevalent. Increased handling and low bacterial counts seem to favor the development of oxidized flavor. Outbreaks of oxidized flavored milk have been particularly intensive in dairies producing high quality milk--especially if the interval between production and consumption is longer than usual.

Oxidized flavor has been studied rather extensively during the last ten years. Out of these studies has come the knowledge that it is a developed off-flavor that may be catalyzed in various ways such as: exposure to light rays, metal contamination, and heat. In accordance with these investigations, plant operators have taken special precautions in handling milk. Little trouble has been reported during the seasons of the year when pasture and other green feeds are plentiful. However, during the winter season some milk develops oxidized flavor when produced under the most sanitary conditions and with the greatest protective efforts against metal contamination and light. Extreme precautionary measures during the processing and handling operations does not always prevent or eliminate the oxidized flavor from milk. The production of milk which is non-susceptible to oxidation may be a possible solution of the problem. Therefore it seemed desirable to study the raw milk from individual cows in an effort to determine the possible relationship of certain milk constituents to the oxidation process.

REVIEW OF LITERATURE

Oxidized flavor may not occur regularly in the milk, and it is not a serious problem to all milk producers but from a review of the literature on this subject it is evident that its occurrence is not infrequent in some herds. From 155 cows in five herds, Guthrie and Brueckner (13) found 21 per cent giving milk that developed a distinctly oxidized flavor. Out of 349 samples of milk entered in the California State Fair, 1930 to 1934, 24.2 per cent were criticized as having an oxidized flavor, according to

Roadhouse and Henderson (23). Roland, Sorenson, and Whitaker (24) state that 21 per cent of 139 samples of commercial pasteurized milk from dairies in 19 different cities had oxidized flavor. Chilson (5) reported that 25 to 30 per cent of the cows in the Cornell University herd gave milk during the winter and spring months that developed oxidized flavors.

In studying the causes of oxidized flavor investigators have discovered various activators that have a catalytic effect on the oxidation process. Freshly drawn milk does not tend to become oxidized unless an activator is present, according to Tracy, Ramsey, and Ruehe (32). Of all the activators that have been studied there are three that appear to have the most vigorous chemical or catalytic effect on oxidation. These are--the action of dissolved metals, oxidation of an enzyme, and the catalytic influence of radiation from solar and artificial light.

In studying the action of metals on oxidation Quam (22) found that copper, nickel, and zinc were the most soluble metals in milk, and therefore the most active. The solubility of these metals increased when the milk was heated. Aluminum and tin were insoluble up to 70° C. while iron and chromium remained insoluble even at this temperature. According to Guthrie, Roadhouse, and Richardson (14), oxidized flavor is usually due to metal corrosion. Their studies showed that copper and copper alloys gave the most pronounced off-flavor. These results are supported by Thurston (27), who reported that oxidized flavor was developed by adding as little as 0.5 parts per million of copper sulphate to milk. He further observed that salts of tin and aluminum did not cause oxidized flavor in milk, and concluded that copper and iron are the only metals capable of causing this flavor defect.

Kende (20) has associated the action of metal salts with an oxidative enzyme, stating that metals act only in the presence of an organic ferment--oleinase. Gondos (12), in 1934, claimed that oleinase is one of the factors that operates to affect oxidation. He claimed that dairy products may be contaminated with heavy metals which increase the activity of oleinase.

In a more recent investigation, results obtained by Chilson (5) support the theory that an oxidizing enzyme is present in milk. In his work, milk which usually developed oxidized flavor was heated to 170° F. for 10 minutes with the result that no oxidized flavor developed. Skimmilk heated in the same manner and added to the previously separated raw cream to form reconstituted milk gave similar results. Chilson concluded that the enzyme, affecting oxidation, is in the skimmilk phase. This conclusion has recently been substantiated by Dahle and Palmer (8).

Perhaps even more effective than the combined action of metal and enzymes, in causing oxidized flavor, is the influence exerted by light. As early as 1920 Hammer and Cordes (15) found that milk and cream exposed to sunlight in ordinary glass bottles yielded a fat lighter in color than the unexposed product. Off-flavors were observed in certain lots of milk after only 10 minutes exposure. This off-flavor could be prevented by the use of brown-glass bottles. Holm, Greenbank, and Deysher (17) reported that the induction period of oxidation is greatly decreased by exposure of the fats to ultra-violet light. According to Davies (9) solar ultra-violet or artificial light radiations are pro-oxygenic factors to the extent to which they can penetrate into the body of a fat. Davies (10) found that if milk with finely dispersed fat was exposed to the radiations of a small mercury vapor lamp for ten minutes the flavor became so bad as to render it almost undrinkable. Corbett (7) showed that there is a rapid shift of the oxidation-reduction potential toward the oxidation side when milk is exposed to sunlight. Roadhouse and Henderson (23) reported that fat oxidation may occur in the milk processing plant if the rays of the sun are allowed to come in contact with it during the pasteurization process or during cooling. They claimed that sunlight has an even greater effect than the action of metals.

The frequent occurrence of oxidized flavor in high grade milk has led to the investigation of the bacterial count in relation to this flavor defect. Roland, Sorenson, and Whitaker (24) found that bacterial counts in milk having oxidized flavor were generally lower than the counts in milk free from this off flavor. Thurston and Olson (31) observed that pasteurized milk stored at 36° to 56° F. had a tendency to develop oxidized flavor, but that this flavor was more evident at lower storage temperatures. They suggested that this offers evidence that the growth of bacteria inhibits the development of oxidized flavor since less growth occurred at lower than at higher temperatures. According to Tracy, Ramsey, and Ruehe (32) the incubation of either raw or pasteurized milk at 80° F. or at 68° F. for a few hours greatly retards or prevents the development of oxidized flavor. The addition of live yeast cells to the milk produced the same effect. Similar results were obtained by Dahle and Palmer (8) when milk was incubated at 98° F. Davies (9) stated, "When milk is stored, the dissolved oxygen is used by the micro-organisms for respiratory purposes, and the rate at which the oxygen is removed is roughly proportional to the number of organisms per unit volume." Frazier and Whittier (11) reported that bacteria and yeast result in a change of the oxidation--reduction potential towards the reduction phase. They concluded that milk of a very good quality, from a bacterial standpoint, is more likely to become oxidized than milk more highly contaminated. Furthermore they claim that this explains why winter milk becomes oxidized more readily than summer milk since bacterial growth is less in the winter. In contradiction to this belief Webb and Hileman (33) found that summer milk is able to resist development of oxidized flavor even in the presence of a high oxidation--reduction potential. They found no relation between the oxidation--reduction potential of milk from individual cows and oxidized flavor. However, the development of oxidized flavor in mixed milk, by the addition of copper, is accompanied by an increase of the oxidation--reduction potential to a point sufficiently high to bring about a change in some milk constituent.

Evidence has been presented by Thurston, Brown, and Dustman (29) that lecithin rather than the fat itself is the constituent oxidized when milk develops this off-flavor. These investigators found that after removal of the absorbed film from the fat globules by washing, no oxidation was obtained in the reconstituted milk even when copper was added. In a later investigation, Thurston, Brown, and Dustman (30) subjected milk to various physical treatments, namely, homogenization, agitation, and alternate freezing and thawing. Milk, after these treatments, was less susceptible to oxidation. They concluded that such treatments caused a movement of the lecithin from the fat globules to the plasma where it is seemingly not oxidized. In support of this work Thurston and Barnhart (28) found that after purified lecithin obtained from dried buttermilk became oxidized it imparted a typical oxidized flavor when dispersed in skimmilk.

Some investigators have reported a possible relation between oxidized flavor and vitamin C in milk. Chilson (6) reported that all the vitamin C in milk was destroyed by the time oxidized flavor was detectable. By adding vitamin C to susceptible milk at the rate of 60 mgms. per liter no oxidized flavor was detected until the seventh day whereas it had previously become pronounced within three days. In a later investigation Sharp, Trout, and Guthrie (25) found no relation between the original vitamin C content of fresh milk and the development of oxidized flavor during storage of milk. A definite relation was found however, when milk was pasteurized, then stored for three days, between the rate of disappearance of vitamin C and the development of oxidized flavor. When milk was pasteurized at a temperature of 170° F. for 10 minutes very little vitamin C was lost and no oxidized flavor developed during storage even though copper was added. According to Sharp, Trout, and Guthrie (25) this phenomenon supports the theory that an enzyme, which is destroyed by high pasteurization temperatures, catalyzes the oxidation of both milk fat and vitamin C. Brown, Thurston, and Dustman (4) found that the susceptibility of milk to oxidation was greatly reduced by supplementing the dry herd ration with a source of vitamin C. The supplements used were tomato juice, lemon juice, and ascorbic acid.

According to Guthrie and Brueckner (13) there is no relation between breed, period of lactation, or age of the cow and oxidized flavor. However, Stebnitz and Sommer (26) found that milk from Holstein cows was more susceptible to oxidation than milk from any other breed, when on winter rations. The milk fat from Guernsey cows had an exceptionally high stability value or peroxide number.

There is a possibility that the change in summer milk which renders it less susceptible to oxidation is produced by a change in feeding conditions. Some of the earliest work relating to feed and oxidized flavor was done by

Kende (20) in 1932. His theory for the absence of oxidized flavor from summer milk was that green feeds and fresh hay contain considerable amounts of reducing substances which tend to prevent oxidized flavor. Furthermore that green feeds seem to lose this reducing substance during storage because of auto-oxidation. In agreement with Kende's observation Brown, Thurston, and Dustman (4) found that by changing cows from dry feed to dry feed plus pasture the milk became non-susceptible to oxidized flavor.

Work done by Anderson (1) has shown that carotene may be the substance in green feed which retards the development of oxidized flavor. He found that by supplementing the regular winter ration with 5 pounds of carrots or 5 pounds of machine cured hay, oxidized flavor could be eliminated from the milk of a cow that had previously produced oxidized milk. Since these feeds are high in carotene he attributed the improvement in flavor to this substance.

The only indication found by Guthrie and Brueckner (13) of a relationship between the feed of the cow and oxidized flavor was the tendency for most cows which produced such milk in the winter not to produce milk that acquired this off-flavor in the summer. They concluded that dry feed was not the sole cause of the development of oxidized flavor since there was a variation in intensity of off-flavor developed in the milk from different quarters of the udder.

Stebnitz and Sommer (26) found that the stability value of fat toward oxidation decreased when cows went on green grass as compared with winter rations. According to their results fat from cows receiving grass as part of their ration is less saturated and therefore more susceptible to oxidation. Yet they found that the milk did not oxidize when the cows were on green feed. These investigators concluded that there are protecting substances in milk in increased amounts when cows are on grass which prevents the development of oxidized flavor. They were unable to find any relationship, however, between the carotene in the milk as evidenced by color of fat and the stability of milk toward oxidation.

EXPERIMENTAL PROCEDURE

This study was undertaken to obtain more information on the occurrence of oxidized flavor in raw milk and its possible relation to various milk constituents. It was planned to study the occurrence of the defect in relation to vitamin C, lecithin, and carotene, as indexed by color of the milk fat. Previous studies had been made with the above constituents, but it seemed that further investigation was needed, especially in connection with carotene. Since the enzyme theory of oxidation had aroused so much interest it was decided to make observations on the amount of phosphatase in relation to oxidized flavor. This enzyme was chosen because it was being investigated in connection with another experiment and preliminary results had indicated a possible relationship.

Milk from four breeds of dairy cows in the college herd (60-70 head) was studied in this trial. Samples of milk from each cow were collected monthly on three consecutive days. Samples of the morning milk were taken immediately after milking, and placed in clear glass bottles. These samples were divided,one part being scored for flavor and tested for vitamin C and phosphatase immediately, while the other part was stored for three days at a temperature of 45° F. The stored sample was examined on the third day for flavor defects and tested for vitamin C. Lecithin and carotene determinations were made on three day composites of the milk.

Cows used in this test were fed a grain mixture along with medium grade alfalfa hay and silage. They did not receive pasture during the trial, except during the month of May. A group of Holstein cows that were on a special feeding trial were included in this study and were regarded as a separate group. Cows in this group differed from cows in the herd in that they were receiving prairie hay and had never received any green feed since they were six months old. This group will be spoken of as E-Holsteins throughout this paper.

Examination For Off-Flavors

The milk after being heated to a temperature of about 90° F. was examined organoleptically by two judges for off-flavors. A system of numbering was used to conceal the identity of the samples from the judges. The intensity of the oxidized flavor was indicated by numbers ranging from one, which was slightly oxidized, to five which was very pronounced. Any samples that were so slightly oxidized as to be questionable were not given a positive score. All other off-flavors were recorded, but were not given a rating as to intensity.

Vitamin C Determinations

Vitamin C determinations were made according to the method developed by Bessey and King (2) and modified by Whitnah and Riddell (34). In these tests 10 ml. of milk stood 30 minutes with 15 ml. of 10 per cent trichloracetic acid. Five ml. of the resulting serum was titrated with

2-6 dichlorophenol-indophenol to a faint pink end point. The results are expressed as milligrams of vitamin C per liter of milk. These tests were made on fresh milk and stored milk for all samples taken during November to March inclusive.

Lecithin Determinations

Horrall's (18) method of determining lecithin in milk products was used in this trial. This method involves the removal of lecithin from milk by Mojonnier extraction of the fat. This extract is heated to a white ash in the presence of magnesium nitrate and the ash brought into solution with HCl and distilled water. The amount of phosphorus in the ash is then determined colorimetrically by comparing the unknown solution with a phosphorus solution of known intensity. From this determination the amounts of phosphorus were calculated in parts per million (p.p.m.). The actual amount of lecithin could be calculated by multiplying p.p.m. of phosphorus with a constant derived from the emperical formula of lecithin (18). However the p.p.m. of phosphorus in an ether extraction of fat is representative of the amount of lecithin in the sample.

Phosphatase Determinations

The method of determining phosphatase was the rough measure originally employed by Kay and Graham (19). A more refined analysis has since been worked out by the same investigators, but for the purposes of this trial the rough estimate is sufficiently accurate. In this method the amount of phosphorus liberated from sodium glycerol phosphate when incubated for three hours in the presence of dilute milk is taken as an index of the amount of phosphatase in the milk. The incubated solution is centrifuged, treated with molybdate, then compared in a colorimeter against a standard solution of magnesium phosphate. The units are expressed as micrograms of phosphorus liberated in one cubic centimeter of incubated solution.

Carotene as Estimated by Fat Color Determinations

The method used in this trial for determining the amount of color in milk fat was developed by Whitnah* and used as a relative index of carotene. In this method a quart of milk was allowed to stand at about 40° F. until

*Unpublished date developed by C. H. Whitnah of the department of dairy chemistry, Kansas State College.

a cream layer formed. After syphoning out the skimmilk the cream layer was removed, placed in a centrifuge tube, and whirled at 2000 r.c.f. for 30 minutes. The concentrated cream layer was then scraped off the tube and churned with an equal amount of sugar for 1-2 minutes. This mixture was then melted and washed into a separatory funnel with hot water. The washing was continued until the wash water became clear. The washed fat was then dissolved in low boiling petroleum ether and allowed to stand until clear. This clear fat solution was matched in a Klette colorimeter against a standard 0.1 per cent potassium dichromate solution.

The concentration of fat in the sample was determined by evaporating the ether solvent from one c.c.of solution and weighing the remaining fat. In calculating the color score the assumption was made that color intensity is proportional to both the concentration of fat in the solution and to the concentration of pigment in the fat. The color scores are calculated by the following formula:

$$\text{Color} = \frac{\text{depth of dichromate}}{\text{Depth of fat solution} \times \text{wt. of fat in 1 c. c. solution}}$$

The assumption that this color determination is an index of carotene is only approximate since not all of the color in milk fat is due to carotene. However it has been reported by Palmer and Eckles (21) that practically all of the color in milk fat is due to carotene so the results obtained are sufficiently accurate to give comparative values among different samples of milk.

Carotene Supplements in The Ration

A previous investigation by Anderson (1) has indicated that oxidized flavor could be improved by adding carrots and dehydrated alfalfa hay to the ration. Since these two feeds are high in carotene the improvement in flavor was attributed to this factor. Only one cow was used in the above work, however, so it seemed desirable to see if the results could be repeated with a larger group of animals. Two feeds were selected that rank high in carotene content. One was a carotene concentrate, soluble in fat solvents, and the other was dehydrated green oats. The carotene concentrate contained 150 mg. of carotene per pound while the dehydrated oats contained 103 mg. of carotene per pound.

Two feeding trials were conducted supplementing the regular winter ration of animals that had been producing badly oxidized milk with the high carotene feeds. In the first trial three cows were fed 2 pounds per head daily of dehydrated oats and a fourth cow received 4 pounds daily of the fat soluble concentrate. In the second trial which came later two cows were fed the fat soluble concentrate at the rate of 1 pound per head daily. Control groups of equal numbers of cows were carried along without any carotene supplement in both trials. Both trials lasted ten days. During the trials, samples of milk were collected daily and examined for flavor defects and analyzed for color.

EXPERIMENTAL RESULTS

Preliminary Study

Only a few samples of milk examined in the November herd test developed an oxidized flavor and when present its intensity was slight. Therefore it was decided to study several factors which might intensify the development of oxidized flavor since a more intense off-flavor would be helpful in studying possible causes of this defect. A group of 16 cows was selected, six of which had previously produced milk that developed an oxidized flavor. The milk from each cow was divided into four parts--part one, untreated raw milk was used as a control; 10 p.p.m. of Cu ($CuSO_4$) were added to part two; 10 p.p.m. of Cu were added to part three which was then pasteurized at 143° F. for 30 minutes; part four was exposed to direct sunlight for

20 minutes then held at 40° F. for 24 hours and examined for flavor defects. The milk was examined for flavor when one, two, and three days old in each case excepting the sample that had been exposed to sunlight.

The results, shown in table 1, indicate that both sunlight and dissolved copper will intensify the development of oxidized flavor. Eleven of the sixteen samples esposed to sunlight developed a distinct oxidized flavor, while two more were very slight. Of the samples to which copper was added, only seven of the raw milk and five of pasteurized developed oxidized flavor. Five of the untreated samples developed oxidized flavor which was less intense than that of the treated samples. Three of the sixteen samples were not affected by any of the treatments.

No further work was done with copper or sunlight treatments since the next herd test revealed an increased amount of oxidized flavor in untreated milk.

Table 1. The occurrence of oxidized flavor* in raw milk, raw milk plus copper, raw milk plus copper followed by pasteurization, and milk exposed to sunlight

Cow No.	Previous flavor rating	Raw: Initial	Raw: 3** days	Raw plus 10ppm. Cu: Initial	Raw plus 10ppm. Cu: 3 days	Pasteurized plus 10ppm. Cu: 1 day	Pasteurized plus 10ppm. Cu: 2 days	Pasteurized plus 10ppm. Cu: 3 days	20 minute exposure to sunlight: 1 day
1	2	1	2	2	3	2	3	3	1
2	1	-	1	-	1	-	1	2	2
3	1	-	-	-	-	-	-	-	-
4	1	-	1	-	1	-	-	1	2
5	2	-	2	-	-	-	1	2	2
6	2	-	-	-	-	-	-	-	1
7	-	-	-	-	1	-	-	-	1
8	-	-	-	-	1	-	-	-	2
9	-	-	1	-	2	-	-	1	2
10	-	-	-	-	1	-	-	-	1
11	-	-	-	-	-	-	-	-	1
12	-	-	-	-	-	-	-	-	1
13	-	-	-	-	-	-	-	-	1
14	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	1

* Oxidized flavor score rating in intensity from 1-5.
** Number of days storage at 40° F. after treatment.

Monthly Flavor Results

The results of the monthly flavor analysis of milk are presented in table 2. This table shows the total number of samples analyzed each month and the per cent of samples developing oxidized and other off-flavors. Off-flavors listed other than oxidized are : rancid, salty, feedy, and storage. No certain relation was found between any other off-flavor and a flavor that could be definitely characterized as oxidized.

Oxidized flavor was detected in 11.02 per cent of 1134 samples examined during the regular monthly herd test (table 2). There was a decided decrease in this flavor defect during the month of May. This improvement in flavor corresponded with the time when the cows received their first pasture after having been fed a dry winter ration for six months.

Table 2. The per cent of milk samples, collected from November to May, that developed oxidized and other off-flavors

Month	Samples	Per cent of off-flavored samples after 3 day storage				
		Oxidized	Rancid	Salty	Feedy	Storage*
Nov.	56	14.3	1.8	0.11	0.04	0.04
Dec.	62	25.8	3.2	0.05	0.05	0.05
Jan.	201	22.4	22.4	0.09	0.14	0.04
Feb.	201	7.5	12.9	0.10	0.04	0.07
Mar.	198	11.1	26.8	0.09	0.05	0.10
Apr.	204	7.4	14.7	0.08	0.11	0.11
May	212	1.9	6.1	0.09	0.13	0.11
Total	1134	11.02	15.0	0.09	0.09	0.08

* Off-flavors such as high acid, stale, musty, sharp.

Relation of Breeds to Oxidized Flavor

The occurrence of oxidized flavor in the milk from the Guernseys and Jerseys was less frequent than that in the milk from Ayrshires and Holsteins (table 3). There were only slight differences between the Jersey and Guernsey breeds and between the Ayrshire and Holstein breeds in

Table 3. The per cent of milk samples, collected from each breed, that developed oxidized flavor

Month	E- Holsteins		Holsteins		Ayrshires		Jerseys		Guernseys	
	No. samples	Per cent oxidized	No. samples	Per cent oxidized	No. samples	Per cent oxidized	No. samples	Per cent oxidized	No. samples	Per cent oxidized
Nov.	9	12.5	13	23.0	12	16.6	13	0	11	18.1
Dec.	9	0	14	35.7	11	27.3	13	7.7	12	33.3
Jan.	30	0	42	35.7	39	23.1	48	14.6	42	21.4
Feb.	36	5.5	39	20.5	39	5.1	48	6.3	39	2.0
Mar.	32	15.6	50	12.0	35	22.9	48	8.3	38	2.6
Apr.	38	7.9	46	8.6	27	18.5	48	2.1	36	0
May	45	3.3	48	2.1	30	13.3	45	0	39	0
Total	199	6.03	252	16.7	193	17.1	263	6.1	217	7.8

occurrence of this flavor defect. The E-Holstein cows were as free from the oxidized flavor as the Jersey and Guernsey breeds. The milk produced by this group, however, frequently became so rancid that other off-flavors might have been indistinguishable.

Relation of Vitamin C to Oxidized Flavor

According to results presented in table 4 there is a difference in the vitamin C content of the milk from different breeds. The rank of breeds from high to low vitamin C is as follows: Jersey, Guernsey, Ayrshire, and Holstein. The breeds ranking highest in vitamin C were also lowest in percentage of oxidized flavor. This gives the appearance that oxidized flavor does not occur as frequently in the milk of a high vitamin C content as in the low vitamin C milk.

Correlation coefficients presented in table 4 show the relationship existing between vitamin C and oxidized flavor by breeds and for all samples. These coefficients were computed by the Product-Moment method using vitamin C in p.p.m. as compared with an oxidized flavor score of 0-5. A correlation coefficient of 1.0 means a positive correlation and of -1.0 a negative correlation. The coefficients in table 4 indicate no significant relationship between either the original vitamin C or the loss in vitamin C during storage and oxidized flavor. The correlation coefficients between original vitamin C and oxidized flavor for 732 samples was only -.017. The coefficient between loss of vitamin C and oxidized flavor was .109, which though slightly higher was still insignificant.

Table 4. The relation between the occurrence of oxidized flavor and vitamin C--the amount of vitamin C in fresh milk, and the amount of vitamin C lost during 3 day storage

Breed	No. samples	Per cent samples oxidized	Mean Vitamin C p.p.m. Original	Mean Vitamin C p.p.m. Loss	Mean Vitamin C p.p.m. Per cent loss	Correlation coefficient Original	Correlation coefficient Loss
Jer.	170	8.8	24.30	10.76	44.3	.042	.017
Guer.	142	12.7	24.03	8.93	37.1	-.003	.010
Ayr.	141	19.1	19.50	9.52	48.8	.142	.016
Hols.	165	22.4	18.37	8.14	44.3	.067	.003
E-Hols.	114	7.0	18.92	7.91	41.8	.009	-.009
Herd Milk	732	14.3	20.38	9.12	44.7	-.017	.109

Relation of Lecithin to Oxidized Flavor

The data on lecithin content, expressed as parts per million of phosphorus, and flavor are presented in table 5. A study of this table reveals that the occurrence of oxidized flavor is only slightly more frequent in the lower lecithin intervals. In comparing the lowest, the middle, and the highest intervals, oxidized flavor occurred in 23.4, 23.1, and 20.0 per cent of the samples falling in those respective

Table 5. The occurrence of oxidized flavor in milks grouped according to lecithin content

Class interval p.p.m. phosphorus	No. of samples			Per cent of total			Per cent of interval total		
	Oxidized	Not oxidized	Total	Oxidized	Not oxidized	Total	Oxidized	Not oxidized	Total
39.5- 69.5	11	36	47	47.8	37.1	39.2	23.4	76.6	100.0
69.5- 99.5	4	15	19	17.4	15.5	15.8	21.1	78.9	100.0
99.5-129.5	3	19	22	13.0	19.6	18.3	13.6	86.4	100.0
129.5-159.5	3	10	13	13.0	10.3	10.8	23.1	76.9	100.0
159.5-189.5	1	9	10	4.4	9.3	8.3	10.0	90.0	100.0
189.5-219.5	0	4	4	0	4.1	3.3	0	0	0
219.5-249.5	0	0	0	0	0	0	0	0	0
249.5-279.5	1	4	5	4.4	4.1	4.2	20.0	80.0	100.0
39.5-279.5	23	97	120	100.	100.	100.	20.5	79.5	100.0

intervals. This does not appear to be a significant difference since 20.5 per cent of all the samples had the defect.

The correlation coefficient between lecithin, expressed as phosphorus in parts per million, and the oxidized flavor score is .0056 for 122 samples. Such a low coefficient does not indicate a significant relationship.

Relation of Phosphatase to Oxidized Flavor

At the beginning of this trial monthly phosphatase determinations were being made in connection with another experiment. Since these results were available and the determinations were being made on samples of milk used in this trial, it seemed desirable to consider this enzyme in connection with the oxidized flavor results. However, no significant relation was found between the amount of phosphatase and the development of oxidized flavor. In 890 samples of milk the correlation coefficient was only .048.

Effect of Carotene Supplements in The Ration on Oxidized Flavor

In the first feeding trial eight cows were selected that had been consistently producing milk which developed an oxidized flavor. Four of this group were fed a carotene supplement in addition to the regular winter ration. The other four were continued on the winter ration without supplement. Milk samples were collected daily and examined in the regular manner for flavor defects. Cow number 134 received 600 mgms. of carotene daily in the form of a fat solvent soluble concentrate. After having received the supplement two days this cow produced milk that gave no indication of developing an oxidized flavor (table 6, figure 1). Her milk had an exceptionally good flavor after five or six days of supplemented feeding. The other three cows, numbers 370, 130, and 242 received 206 mgms. of carotene per head daily in the form of dehydrated green oats. All of these cows produced milk after the third day of supplemented feeding that did not develop oxidized flavor. Although the oxidized flavor seemed to be eliminated from the milk of these three cows the flavor was not as good as it was for cow number 134 which received the higher amount of carotene. Milk produced on the ninth day of the trial by cow number 130, which was on the lower carotene level, developed a slightly oxidized flavor. This was the only instance in which oxidized flavor returned, after the third day of the trial, in the milk of all four cows. The control group of four cows maintained on an unsupplemented ration produced oxidized milk during the entire feeding trial, figure 1.

A second similar feeding trial was conducted with four animals--two as a control group, and two receiving carotene supplement. In this trial cows 249 and 382 received 150 mgms. of carotene per head daily in the form of fat solvent soluble concentrate. As shown by the results in table 6 and figure 1 the flavor was improved after the second day of carotene feeding. The improvement was not as pronounced in the milk of these cows as it was from the cows in the first feeding trial on a higher level of carotene. Cow number 249 produced milk on the seventh and eighth days with a slightly oxidized flavor. Milk from both cows was slightly off-flavored (stale or musty) during the entire feeding trial. When they were turned out to pasture a decided flavor improvement was noticed. The control group continued to produce milk with an oxidized flavor during the entire feeding trial, figure 1.

No attempt was made to learn how soon oxidized flavor would have returned in the milk of the cows that had been receiving a carotene supplement. All of the animals used in this experiment were put on pasture shortly after the feeding trials ended.

Table 6. Intensity of oxidized flavor in the milk of cows prior to and after feeding carotene supplement

Date	Trial number 1				Date	Trial number 2	
	Cow number					Cow number	
	370	134	130	242		249	382
Mar. 1	-	4	-	-	Mar. 1	5	-
Mar. 2	1	2	-	1	Mar. 2	5	-
Mar. 3	3	1	1	-	Mar. 3	5	-
Mar.20	1	1	-	-	Apr. 3	5	4
Mar.21	-	2	1	1	Apr. 4	3	-
Mar.22	1	1	-	-	Apr. 5	5	-
Supplement started March 22					Supplement started Apr.10		
Mar.23	1	2	3	3	Apr.11	5	3
Mar.24	1	1	1	1	Apr.12	3	1
Mar.25	-	-	3	3	Apr.13	-	-
Mar.26	-	-	-	-	Apr.14	-	-
Mar.27	-	-	-	-	Apr.15	-	-
Mar.28	-	-	-	-	Apr.16	-	-
Mar.29	-	-	-	-	Apr.17	1	-
Mar.30	-	-	-	-	Apr.18	1	-
Mar.31	-	-	2	-	Apr.19	-	-
Apr. 1	-	-	-	-	Apr.20	-	-
Apr. 2	-	-	-	-	Apr.21	-	-
Apr. 3	-	-	-	-	Apr.22	-	-
Apr. 4	-	-	-	-	Apr.23	-	-
Apr. 5	-	-	-	-	Apr.24	-	-

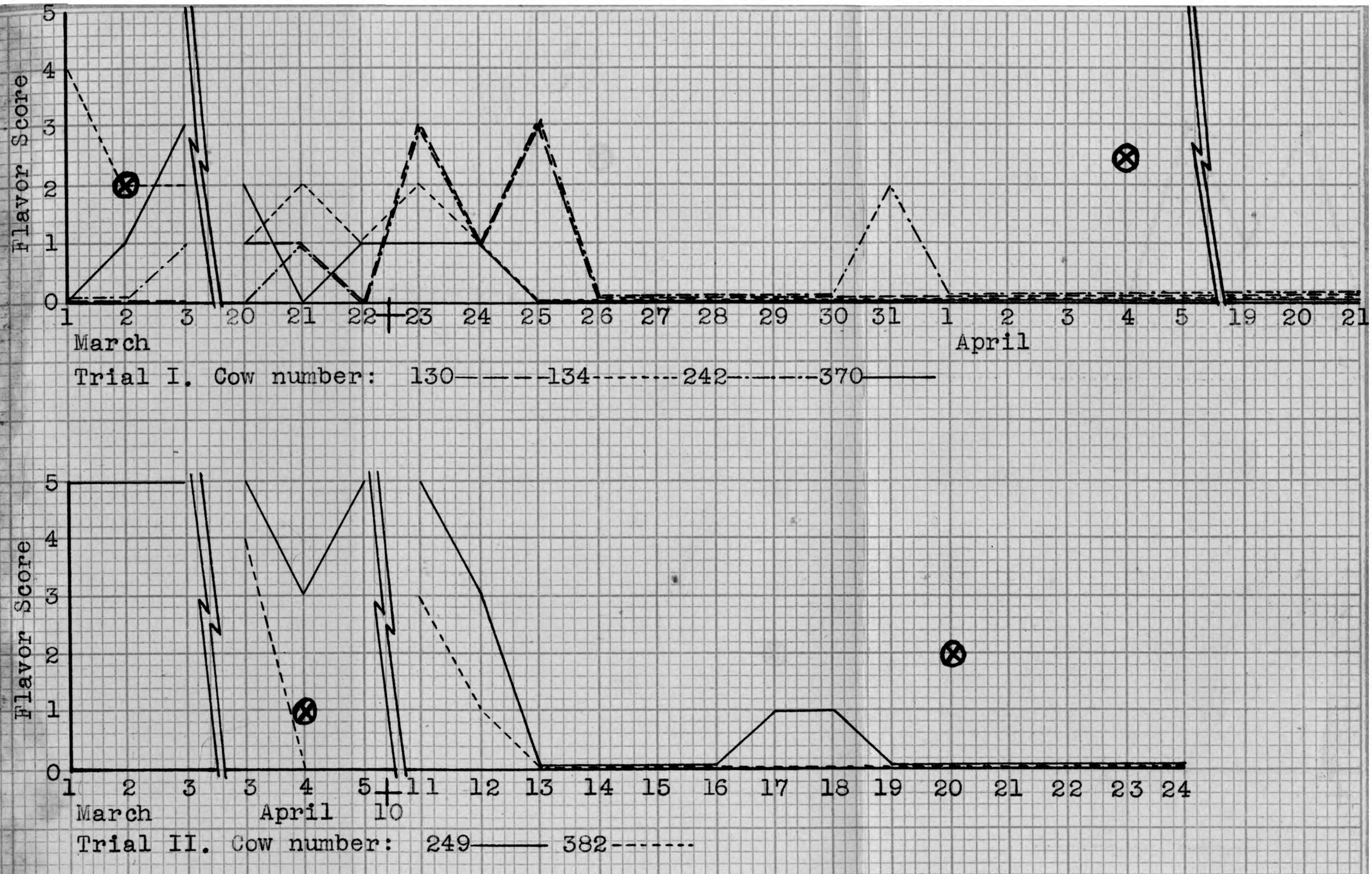

Fig. 1. Effect of carotene supplements in the ration on oxidized flavor.
⊗ Indicates flavor score of unsupplemented controls.
+ Date carotene supplement was started.

The Relation of Milk Fat Color to Oxidized Flavor

The effect of carotene supplements in the ration on color intensity of the milk fat and oxidized flavor in milk is shown in table 7, figure 2. Color determinations were made on the milk from each cow included in the first feeding trial. These determinations are not shown for the cows in the second feeding trial because several of the samples taken at the end of the trial were accidentally destroyed.

According to these results there was an appreciable rise in fat color intensity shortly after the cows had received a carotene supplement. In each case oxidized flavor was eliminated as the color intensity increased. Color intensity was increased as much as 162 per cent above the breed average, cow number 134 figure 2, after a carotene supplement had been fed. This cow received nearly 400 mgms. more carotene per day than the other cows in this group. In the milk of cow number 130, color intensity was not materially affected, but it is significant to note that when the color was increased above breed average oxidized flavor did not develop. The last color determination made on the milk of cow number 130 showed a decrease in color to a score 4 per cent below breed average. This determination was made shortly after the feeding trial had ended thus indicating that this cow had not utilized the carotene as well as the others. That high color intensity, in comparison with breed average, does not always prevent the development of oxidized flavor is shown in the results from cow number 370. Milk from this cow was slightly oxidized when the color intensity was 53 per cent above breed average. However her milk was decreasing in color and the off-flavor was becoming more pronounced as the color decreased. The flavor defect was eliminated entirely after feeding a carotene supplement.

The relation of milk fat color to oxidized flavor is presented in table 8. Since the Holstein and Ayrshire breeds do not have as highly colored milk as the Jersey and Guernsey breeds their results were computed separately. It will be noted that the minimum color score for the non-oxidized flavor groups was even lower than the minimum for the oxidized flavor group. However, the maximum score was about 3 times as high for the non-oxidized flavor groups. Both the mean and the median of the oxidized and non-oxidized flavor groups indicate that this flavor defect occurs more frequently in low color milks than in high color milks. The comparatively wide difference between the mean for the oxidized flavor group and the mean for all samples indicates that in all but a few instances the color intensity of the oxidized milk was below that of the breed average.

Table 7. The effect of carotene supplements in the ration on color intensity of milk fat and the development of oxidized flavor in milk

Date	Cow number											
	370			130			134			242		
	Color score	Per cent of breed average	Flavor	Color score	Per cent of breed average	Flavor	Color score	Per cent of breed average	Flavor	Color score	Per cent of breed average	Flavor
Mar. 2	5.7	45	2	8.4	85	1	10.3	105	1	11.8	153	1
Mar.21	13.0	105	1	9.5	93	1	9.5	93	2	9.4	122	1
Ration supplemented with a carotene concentrate March 22												
Mar.29	18.0	154	-	12.0	113	-	9.3	93	-	7.5	95	2
Apr. 4	14.7	124	-	11.9	112	-	27.8	262	-	10.1	150	-
Apr.20	17.0	145	-	10.2	96	1	-	-	-	8.7	148	-

Table 8. The relation of milk fat color to oxidized flavor

Flavor	Holstein-Ayrshire				
	Color score				
	Minimum	Maximum	Mean	Median	No samples
Not-oxidized	1.9	51.5	13.8	10.8	77
Oxidized	2.6	18.5	7.6	8.3	25
Total	1.9	51.5	12.6	9.2	102
	Guernsey-Jersey				
Not-oxidized	2.7	82.0	24.8	18.4	104
Oxidized	3.7	22.1	11.9	12.5	10
Total	2.7	82.0	21.0	17.7	114

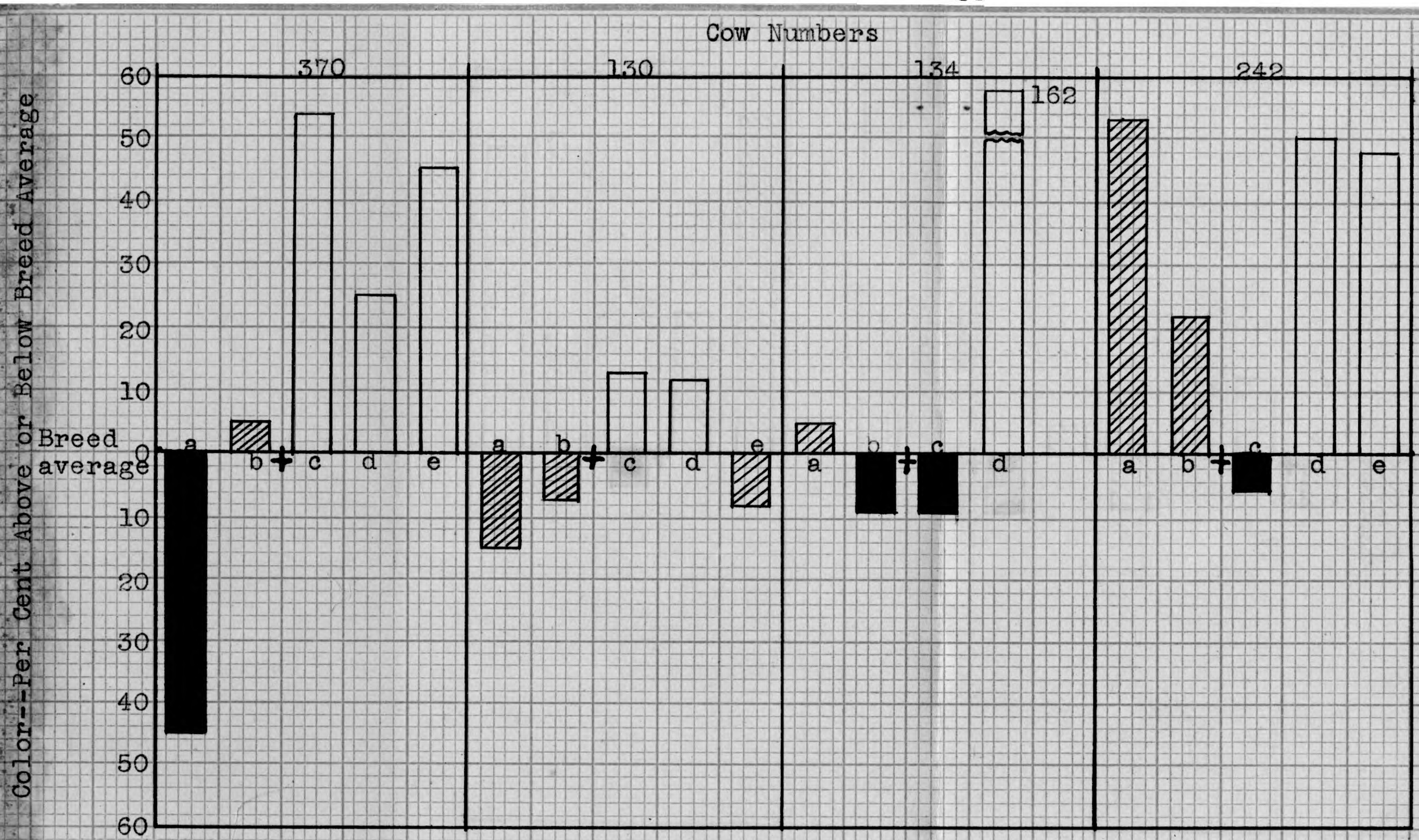

Fig. 2. Effect of carotene supplements in the ration on color and oxidized flavor.

Legend

oxidized flavor
slightly oxidized
not oxidized

a--March 2
b--March 21
c--March 29
d--April 4
e--April 20

+--carotene supplement started March 20

DISCUSSION

Results obtained in this trial seem to indicate that there is a relationship between breed and the susceptibility of raw milk to become oxidized. Guthrie and Brueckner (13) and Dahle and Palmer (8) could find no such relationship, but the results obtained by Stebnitz and Sommer (26) agree with the foregoing results. There was a substantial difference between the Holstein and Ayrshire breeds, and the Jersey and Guernsey breeds. Oxidized flavor occurred in 16-17 per cent of 445 samples of Holstein and Ayrshire milk whereas it only occurred in 6-7 per cent of 480 samples of Jersey and Guernsey milk.

The spontaneous occurrence of oxidized flavor in raw milk does not seem to be affected by the original amount of vitamin C in the milk or the loss of this vitamin during storage. Chilson (6) found that all of the vitamin C was destroyed by the time oxidized flavor was detectable. In this trial however there were instances in which most of the vitamin C was left after 3 days storage and still oxidized flavor had developed. Sharp, Trout, and Guthrie (25) found a definite relationship between the rate of disappearance of vitamin C during storage and the development of oxidized flavor when the milk was pasteurized at 143° F. for 30 minutes. Such a relationship may exist in pasteurized milk but it did not hold true for raw milk. In this experiment there is no relation between the natural amount of vitamin C in milk and oxidized flavor. It seems possible however that this flavor defect could be prevented by the addition of vitamin C to the milk in large enough quantities as was done by Chilson (5) and Sharp, Trout, and Guthrie (25).

From the review of the literature it seemed that lecithin is the substance oxidized when milk develops this flavor. Consequently it would appear that the amount of lecithin in the milk would influence the intensity and frequency of oxidation. In this trial, however, oxidized flavor occurred just as frequently in the lower lecithin intervals as in the highest amounts. Thurston and co-workers (30) found that the physical state of lecithin in the milk, i.e. whether it is surrounding the fat globules or dispersed in the plasma, was a contributing factor in causing oxidation. It would appear that the physical and chemical state of lecithin are more important than the amount, if lecithin actually is a cause of oxidized flavor.

The susceptibility of raw milk from cows on dry winter rations to become oxidized can be eliminated by supplying a carotene supplement in the feed of the cow. A carotene concentrate furnishing 600 mgms. of carotene per head daily proved very effective in preventing oxidized flavor and even improved the flavor of the milk. A carotene concentrate supplying only 206 mgms. of carotene daily seemed to be almost equally effective in preventing oxidized flavor. This seems to be the minimum amount of carotene that will eliminate oxidized flavor in the milk of cows that have been on a ration low in carotene for about four months. When a lower amount of carotene was fed, it decreased the intensity of oxidized flavor, but was insufficient to produce normal flavored milk.

Stebnitz and Sommer (26) were unable to find any relationship between the stability of fat to resist oxidation and the carotene content as evidenced by fat color. Results obtained in this trial seem to indicate that high carotene content of the milk renders milk fat less susceptible to oxidation. First the Jersey and Guernsey breeds produced milk comparatively free from oxidized flavor. It is common knowledge that these breeds put more carotene into their milk than the other breeds. Then there was a decided increase in the fat color of the milk produced by the cows receiving a carotene supplement indicating that the increased carotene was directly or indirectly resposible for the elimination of oxidized flavor. Finally from the general herd observations oxidized flavor occurred much more frequently in the milk of low fat color. It is true that some oxidized samples were rather high in color, but this is logical in view of the many factors known to cause oxidized flavor.

Oxidized flavor seldom occurs during the seasons of the year when the cows are receiving plenty of green feed in their ration. It has also been established that the spontaneous occurrence of oxidized flavor could be prevented in winter milk by feeding the cows a carotene supplement. Therefore it appears that the problem of eliminating oxidized flavor in raw milk is a problem of producing non-susceptible milk. It would seem that this could best be done by maintaining a ration high in carotene throughout the year by the use of carotene concentrates during the winter months.

SUMMARY AND CONCLUSIONS

1. Oxidized flavor occurred in 11.02 per cent of 1134 samples of milk.
2. Some milks did not become oxidized even though exposed to direct sunlight and contaminated with copper. Eighty per cent of the samples exposed to sunlight and forty-four per cent of the samples to which copper was added developed oxidized flavor.

3. Oxidized flavor occurred in 6-7 per cent of 480 samples of Jersey and Guernsey milk and in 16-17 per cent of 445 samples of Ayrshire and Holstein milk.
4. No relation was found between the natural amount of vitamin C in milk or between the amount of vitamin C lost during storage and the development of oxidized flavor.
5. No relation was found between the frequency of occurrence of oxidized flavor and the amount of lecithin.
6. No relation was found between the frequency of occurrence of oxidized flavor and the amount of phosphatase in milk.
7. The development of oxidized flavor in raw milk was prevented by feeding carotene supplements supplying 600 and 206 mgms. of carotene per head daily. The intensity of the off-flavor was retarded by feeding as little as 150 mgms. of carotene per head daily.
8. Oxidized flavor was most prevalent in milk which was below breed average in fat color intensity.

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