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To the Committee on Graduate Study:

I am submitting to you a thesis written by George Christianson entitled "Occurrence and Prevention of Oxidized Flavor in Milk." I recommend that it be accepted for nine quarter hours credit in partial fulfillment of the requirements for the degree of Master of Science with a major in Dairying.

L. C. Gylie

Major Professor

We have read this thesis
and recommend its acceptance:

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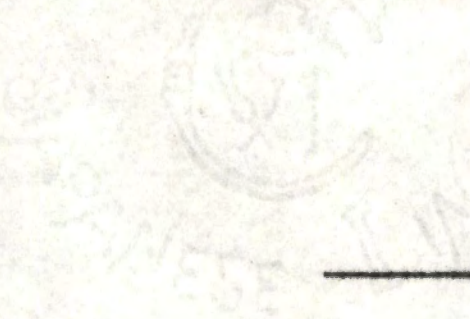
Accepted for the Committee

V. C. Smith
Dean of the Graduate School

OCCURRENCE AND PREVENTION
OF OXIDIZED FLAVOR IN MILK

A THESIS

Submitted to
the Committee on Graduate Study
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science



by

George Christianson

August 1940

PREFACE

The material contained in this thesis has been collected by the writer during a year of graduate study in Dairying at the University of Tennessee.

The writer wishes to express his gratitude to the members of the University of Tennessee faculty for assistance rendered in this study.

Special acknowledgment is due to Professor C. E. Wylie and Professor T. B. Harrison, who gave valuable suggestions at different times, to Mr. H. B. Henderson, who worked with the writer throughout the experiment, and to Lenore Greenway, who typed this thesis.

- George Christianson

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INTRODUCTION

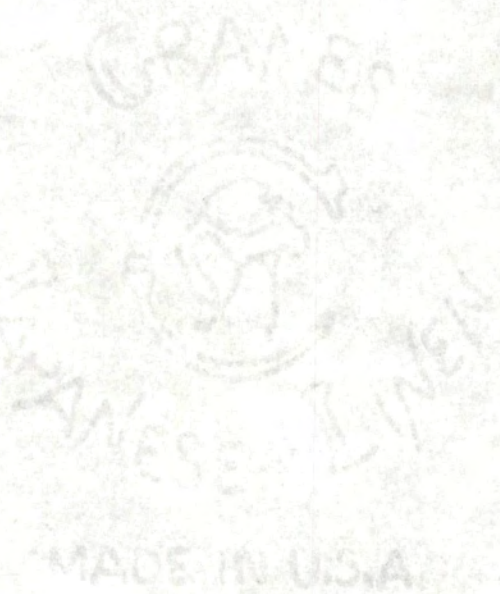
During the past century milk has become one of the most important foods used in the civilized world. The primary reason for this is perhaps due to the increased knowledge of its excellent food value. Sanitary methods and better transportation facilities also have helped to establish it. In the study of milk as a food, three things must be considered; namely, food value, purity, and flavor.

As to the first two considerations, milk can well hold its own with other foods. The phrase, the most nearly perfect food, which is commonly used when speaking of milk, well illustrates the general acceptance of milk as one of the best foods in its nutritional value. Knowledge of herd testing and pasteurization have eliminated any need for impure milk to be sold, and the average consumer now buys milk with a justified confidence. It is true that there are a few cases in which questionable milk is sold, but these cases can not be corrected by further research. They can be corrected only by further education and regulation.

The main problem before the milk industry today is that of flavors. Milk plant operators have been slow to realize this, believing that if the other two qualifications, food value and purity, are fulfilled, their work is done. The opposite is true with the consumer whose first impression of the milk is the taste and smell—this often-times being an important factor in determining the amount that the consumer will use.

Although much more has been done in the field of flavor research than the plant operators have knowledge of, the field is still relatively new and much work remains to be done. Each group of workers in the dairy field has its particular problem to meet. For those interested in herd

management, there are the flavors due to the care of the cattle, for the bacteriologist, the effects of certain strains of organisms, and for the chemist, that large group of flavors that can be attributed only to the chemical breakdown of milk. It is not possible to separate these three into distinct classes, but all are interdependent upon each other.



PART I

REVIEW OF LITERATURE

ORIENTATION

Milk has a mild, pleasantly sweet flavor that appeals to most people, but at times other flavors occur that are not natural and make the milk less palatable. An attempt has been made to classify these flavors. However, at times flavors occur that cannot be explained or put in any one class. In general it can be said that objectionable flavors may be divided into two main classes; those found in the milk at the time of milking and those that develop later.

Most flavors found in milk at the time it is drawn may be attributed to the feed the cow eats or the air she breathes. Roadhouse and Henderson⁽¹⁾ did intensive work to prove this and found that only a short time was required for the flavor to pass from the cow into her milk. In their work they failed to account for the difference of individual cows that were treated at the same time under similar conditions. This would indicate that there are other factors which influence the flavor of milk that have not yet been noted.

After the milk has been drawn, off flavors may develop due to contact with foreign materials, bacterial action, or chemical changes in the milk. Those flavors that are caused by contamination by foreign materials (e.g. kerosene or boiler steam) are not the problem of the research worker, but rather of the plant operator who should seek to remove these sources of contamination.

That flavors develop in milk due to the action of bacteria is usually well known to the consumer. In due time he expects the milk to sour. In addition bacteria may cause other defects in milk such as cheesy, gassy, malty, stringy, etc. However, milk is usually kept at

relatively low temperatures so that bacteria do not grow rapidly and the problem is thus minimized.

Flavors that develop due to a chemical breakdown of the milk are not as readily recognized and offer one of the greatest problems in fluid milk industry. The breakdown of the components of milk are, in some cases, due to enzymes. Rancid flavor is one that several workers (2) (3) (4) claim is due to the lipase, or fat splitting enzymes, found in milk. Oxidized flavor is also due to some chemical reaction involving some part of the milk, but it is doubtful if there is an enzymatic influence.

Occurrence. Nelson and Dahle⁽⁵⁾ reported, "Oxidized flavor is recognized as the most serious defect in fluid milk." This statement was confirmed by Roland et al.⁽⁶⁾ in a study on the quality of commercial pasteurized milk. They also noted that premium milk was more likely to have this defect. Martin⁽⁷⁾ examined many samples of commercial pasteurized milk and reported that ten to twenty per cent of the milk was criticized for being oxidized. It is also interesting to note that this difficulty is mainly encountered in market milk and that, according to Tracy,⁽⁸⁾ no cases have been reported in condensed or evaporated milk. Corbett and Tracy⁽⁹⁾ later made a similar observation.

Consumption. Oxidized flavor is significant because of its economic effect on the industry. If the consumers, especially children, do not like the flavor of milk they will not drink as much of it. The effect is twofold. First there will be less consumption of milk immediately, and second, some consumers will shift to using other products and

will not use as much milk even after the objectionable flavor is no longer present.

Definition. The oxidized flavor in milk is difficult to define because it is different from other flavors. It has been described as being cappy (i.e. the sensation produced by licking a milk bottle cap), tallowy, cardboard, and metallic. The metallic flavor encountered is similar to that sensation given to the tongue by a tarnished piece of copper. These four flavors are now recognized as being the same, the only variance being due to the intensity of oxidation. Nelson and Dahle⁽⁵⁾ have suggested a possible nomenclature for these varying intensities. From "astringent", which is the first indication, they proceed through "papery", "metallic", and "metallic and oily" to the most intense or "tallowy". Since at the present time the only means of identifying this flavor is by taste, it is likely that such a proposed standard will be adopted. Until this is done it will be difficult to compare the results of others and it will be more difficult to check the work between laboratories. As yet any other method that would eliminate the human element and lessen the chance for error has not been found.

Scope. Oxidized flavor does not develop in all milk and is dependent on several factors. In the words of Barkworth,⁽¹⁰⁾

Oxidized flavor in milk may be induced by copper contamination, but some milks are more susceptible to oxidation than others. Moreover it is possible for the flavor to develop spontaneously. Diet of the cow plays a part both for and against oxidation. Green feeds tend to yield a more stable milk. Since the milk serum appears to carry the activity factors and since these are apparently heat labile, it is believed that enzymes are involved. Exposure of milk to sunlight catalyzes the oxidation of the fat.

Nelson and Dahle⁽⁵⁾ and Thurston⁽¹¹⁾ also mention the effects of light and copper as well as the varying susceptibility of different milks.

In the light of these and other observations, Thurston⁽¹²⁾ raised the following questions as possible keys to the problem:

1. Is the milk constituent that is oxidized the lecithin, the butterfat, or some other compound in milk?
2. What is the "mechanism" affected by high heat treatment?
3. Is an enzyme concerned in the development of oxidized flavor?
4. In what manner do agitation, freezing and thawing, and homogenization have their effects?
5. Why does copper added to milk before pasteurization have less effect in the production of oxidized flavor than copper added after pasteurization?
6. Why are some milks susceptible to the development of oxidized flavor following the addition of copper whereas other milks are not susceptible while still other milks develop oxidized flavor without the addition of copper?

Since Thurston raised these questions some of them have been answered, but the problem as a whole has not been solved.

CORRELATIONS

Much work has been done to find what correlation there is between the following variable factors and the occurrence of the oxidized flavor in milk. By a study of these correlations it might be possible to find the cause, and then work could be started on the correction of the difficulty. Factors studied vary from the bacterial quality of the milk to its chemical makeup and the presence of foreign matter such as copper.

Copper. It has long been known that copper in milk has some effect on its susceptibility to becoming oxidized. The first labora-

tory method used to add copper to milk was that described by Roland and Trebler⁽¹³⁾ who used strips of metallic copper. These strips were all cut so that the same surface area was exposed and immersed in the milk as it was being pasteurized. In some cases, the copper was weighed before and after to determine the amounts that had been absorbed. A more satisfactory method of determining the amounts of copper absorbed is the Ritter's test as used by Turgeon, Stebnitz, and Sommer.⁽¹⁴⁾ They also recommended this test to check the susceptibility of milk to become oxidized. Henderson⁽¹⁵⁾ and Brueckner⁽¹⁶⁾ also reported their finding that copper induced oxidation of milk. Brown, Thurston, and Dustman⁽¹⁷⁾ used copper sulfate solution to add known amounts of the metal and in that way were able to determine more nearly the effect of increasing copper contamination. They concluded that up to 1.3 p. p. m. the more copper added the more susceptible the milk would be, and the stronger the developed flavor. However, adding more than this did not increase the flavor development.

In 1939 Roadhouse⁽¹⁸⁾ compared strips of metallic copper to certain alloys to determine if more or less copper would be absorbed. The results indicated that milk absorbs more copper from the pure metal than from alloys containing zinc or tin. As would be expected, the milk that absorbed the most copper developed the strongest oxidized flavor. From this lead Roadhouse and Henderson⁽¹⁹⁾ did further work in attempting to find an alloy that would be suitable for dairy plants and eliminate copper absorption. They found that those alloys containing tin and zinc were the most suitable for the dairy industry in respect to casting,

fitting, wearing quality, and ease in cleaning, as well as to copper contamination.

Brown, Thurston, and Dustman⁽²⁰⁾ reported that milk contaminated with copper after pasteurization was more active than that added before pasteurization and that copper added to raw milk developed oxidized flavors at about the same rate as copper added to milk after it had been pasteurized.

Light. It has long been realized that light has a catalytic effect in the development of an off flavor in milk that is similar to that caused by copper. Nelson and Dahle⁽⁵⁾ compared this flavor to the oxidized flavors caused by the addition of copper and those that developed spontaneously, and concluded that they were all the same. They did not give any possible reason why these three should be the same, but did suggest a possibility of an oxidation of the fat.

Cows. Brown, Thurston, and Dustman⁽²¹⁾ found that there is a great difference in the milk from different cows. Some gave milk that developed an oxidized flavor spontaneously while others gave milk that never became oxidized even with the addition of copper. In 1938 Hening and Dahlberg⁽²²⁾ reported that heifers seemed to give milk that was more susceptible to oxidation and indicated that an investigation would be made along that line. The results of this experiment were published a year later.⁽²³⁾ Only the cows of the college herd were included in this experiment and of this group only six were heifers. In this case the milk of the heifers was much more susceptible to oxidation than the milk from the cows. However, since there were so few animals in the experi-

ment, the authors would not make a definite conclusion.

The breed of the cow may also be an important factor since Beck, Whitnah, and Martin⁽²⁴⁾ noted significant differences in the milk that they studied. In 928 samples of milk studied, oxidized flavor was found in 6.1% of the Jersey, 7.8% of the Guernsey, 15.8% of the Holstein, and 19.4% of the Ayrshire milk. Since only fifty to sixty cows were used, these data cannot be taken as conclusive.

Feed. It is generally agreed that the feed of the cow has some effect on the milk. It may be that some compound in the feed diffuses from the stomach of the cow into the milk, which later is the cause of the oxidized flavor. Although the work of Davies⁽²⁵⁾ with trimethylamine oxide did not deal directly with oxidized flavors it did illustrate how compounds in the feed diffuse into the milk. Further, he found that this oxide did not give an offensive flavor, but later, on breaking down, it made the milk rancid.

Stebnitz and Sommer⁽²⁶⁾ observed that cows on poor rations gave milk that was generally more susceptible to the development of the oxidized flavor. They also attributed a possible reason for the production of such milk due to the presence of certain unsaturated fatty acids. Although they found the summer milk more stable, they felt that its fat was more susceptible and that it was due to the presence of certain antibodies that the flavor did not develop as readily. Beck, Whitnah, and Martin⁽²⁴⁾ also found that pasture seemed to help prevent the development of oxidized flavor.

The works of Hening and Dahlberg⁽²²⁾ (23) are in direct disagree-

ment with Stebnitz and Sommer⁽²⁶⁾ and indicate that the level of feeding has no effect on the quality of the milk. Such a finding suggests the possibility of the kind of feed being the cause of the difficulty. Both Garrett and Bender⁽²⁷⁾ and Whitnah, Peterson, and Atkeson⁽²⁸⁾ compared milk from cows on varying rations as to its susceptibility to become oxidized. These rations included beet-pulp, corn silage, and A.I.V. silage as well as pasture as the source of roughage. Garrett and Bender⁽²⁷⁾ found the feeding of grass silage lessened the tendency of the milk to become oxidized. Whitnah and coworkers⁽²⁸⁾ found a correlation of oxidized flavor to the carotene level, but contended this was not the only factor.

Because of the wide use of beet-pulp and the seeming relationship of oxidized flavor to its feeding, Henning and Dahlberg⁽²⁹⁾ investigated this, but found that neither mangles nor beet-pulp affected the milk in this manner. Liebscher⁽³⁰⁾ compared the milk of cows fed beet tops with the milk of those fed beet tops and copper sulfate. He found no difference in the copper content or the flavor score of the milk from the two different sources. Thus, in view of such varied results, more work is needed to determine the part that feed plays in the development of oxidized flavor in milk.

Composition. The composition of the milk is another point to consider in seeking a possible correlation that might indicate the reason for oxidized flavor in milk. Early work⁽³¹⁾⁽³²⁾⁽³³⁾⁽³⁴⁾⁽³⁵⁾ indicated that there was some relationship to the amount of vitamin C in the milk to its susceptibility to become oxidized. Bech, Whitnah, and Martin⁽²⁴⁾ in a more recent study contradicted this contention, implying that the

correlation was closer to the vitamin A content and intensity of yellow color. In connection with the vitamin A, Stebnitz and Sommer⁽²⁸⁾ found no relationship to the carotene level and the oxidation of the fat in milk. Although Cave, Martin, and Beck⁽³⁶⁾ fed carotene up to 300,000 international units daily, no improvement was noted in the milk flavor. On the other hand, Garrett, Tucker, and Button⁽³³⁾ claimed that high carotene content in milk is "coincidental with good flavor" and Whitnah, Peterson, and Atkeson⁽²⁴⁾ indicated that there was some correlation to oxidized flavor and low carotene content in milk. Beck, Whitnah, and Martin⁽²⁴⁾ prevented the occurrence of oxidized flavor in milk by the feeding of 206 mgm. of carotene daily. These discrepancies may be due in part to the different feeds used as a source of vitamin A. Fraps and Treichler⁽³⁷⁾ found that feed is the source of vitamin A in milk, but did not try different feeds. Later Hauge, Wilbur, and Hilton⁽³⁸⁾ reported that the feeding of certain substances suppressed the amount of vitamin A in butterfat even though the cow was on a high vitamin A intake.

Much work has been done on the color in respect to the flavor of milk, and all literature available⁽²⁴⁾⁽³³⁾⁽³⁹⁾⁽⁴⁰⁾⁽⁴¹⁾⁽⁴²⁾ has recognized such a correlation. The intensity of the color is measured in the laboratory by a photoelectric spectroscope. Light waves between 375 and 480 millamicros is passed through milk and the amount of absorbed light indicates the amount of yellow color in the milk. The American Guernsey Cattle Club has now perfected a simplified machine to measure this color. It works on the same basic principle, but since it is made only to measure the color of milk, it is not as complex or as expensive as the photoelectric spectroscope. Thus, it is possible for many more laboratories

to make color studies.

It was found by Garrett, Arnold, and Hartman⁽⁴⁰⁾ that the color of the milk was affected by feeding different roughages. Cows fed grass silage produced a much yellower milk than did those fed corn silage or beet pulp. Tucker, Garrett, and Bender⁽³⁹⁾ also observed that pasture increased the yellow color of milk and decreased its susceptibility to become oxidized.

Stebnitz and Sommer⁽²⁶⁾ in their study of the color and carotene content of the milk recognized these as factors and added that there might still be another consideration—the saturation of the fat. In their studies they felt that summer pasture made the milk more susceptible to oxidation, and it was due only to the antibodies in the milk that it was not oxidized. Garrett, Bender, and Tucker⁽³⁸⁾ did not find any relationship to composition of the fat and the development of the oxidized flavor.

Anderson, Dowd, and Stuewer⁽⁴³⁾ found that winter milk of higher acidity was more susceptible to oxidation than summer milk of lower acidity, and concluded that there might be some relationship to natural acidity and occurrence of oxidized flavor. However, when Brown and Dustman⁽⁴⁴⁾ tried to duplicate this work they failed to check these results. They also tried to prevent the occurrence of this flavor by standardizing milk to .13% acidity, but were unsuccessful in this.

Greenbank⁽⁴⁵⁾ claims that there is a close correlation of the oxidation-reduction potential of milk and the occurrence of the oxidized flavor. He further states⁽⁴⁶⁾ that a quick check on the suscepti-

bility can be made on the milk by adding copper sulfate and noting the immediate change in the oxidation-reduction potential. This is a relatively new idea which may prove of value in selecting milk in the future.

Processing. Processing in the milk plant also has a great deal to do with the occurrence of the oxidized flavor. Thurston et al.⁽⁴⁷⁾ did some of the first work in this field. They studied homogenization, agitation, freezing, and thawing. Samples of milk were passed through a single stage homogenizer at pressures ranging from zero to five thousand pounds per square inch. There was sufficient copper exposed in the machine so that milk put through with no pressure developed oxidized flavor, but as the pressure was increased up to five hundred pounds a decrease in the occurrence of this flavor was observed. All milk that was homogenized over three thousand pounds per square inch failed to develop any oxidized flavor.

They also checked the effect of agitation at low temperatures and found that this, too, lessened the occurrence of the flavor. Freezing and thawing also had the effect of lessening the susceptibility. However, the milk treated in this way had a tendency to have a cream plug. The authors felt that the reason for such results was that the lecithin was removed from the fat globule and held in the serum. Roland and Trebler⁽¹³⁾ reported that separation and standardization reduced susceptibility, which again may be linked to the relation of the lecithin to the fat globule.

Corbett and Tracy⁽⁹⁾ noted that condensed milk never developed an oxidized flavor and wondered if the heating of milk in a vacuum pan

would eliminate oxidized flavors. Milk was heated at 130-140 degrees F. at 24 to 25 inches vacuum. Copper was added and air was bubbled through the milk. Under these conditions the oxidized flavor failed to develop if the milk was condensed to half its original volume. Although less condensing did not eliminate the occurrence of this flavor, it was not as prevalent as in unheated milk. Barkworth⁽¹⁰⁾ found that heating the milk to 160 degrees for thirty minutes accomplished the same results. Thus, both⁽⁹⁾ ⁽¹⁰⁾ concluded that an antibody must be formed in the milk at these temperatures.

Josepheson and Doan⁽⁴⁸⁾ did more intensive work along this line.

As a result of their experiments, they stated the following:

When milk, cream, skim milk, and some other dairy products are heated to a sufficiently high temperature or held for sufficient period of time at lower temperatures, sulphhydryl compounds are formed from one or more of the proteins present.

These sulphhydryl compounds seem to be wholly responsible for the cooked flavor of heated milk and milk products. They are also responsible for the decrease in the oxidation-reduction potential noted in heated dairy products, since they are active reducing substances.

The sulphhydryl compounds are active antioxidants and appear to be responsible for the inhibition of the development of tallowy or oxidized flavors in milk heated to temperatures over 170 degrees F.

In becoming oxidized (spontaneously or due to copper) the sulphhydryls apparently lose their flavor characteristics and the milk or milk product which previously exhibited a cooked flavor becomes indistinguishable from similar unheated milk.

In addition, they found that milk heated to these high temperatures retained more of its vitamin C.

PHYSICOCHEMICAL

Enzymes. With the limited material available, several men have attempted to explain the presence of the oxidized flavor in milk. Barkworth⁽¹⁰⁾ on finding that heat prevented the development of this flavor attributed the reaction to enzymatic influences, but no one has confirmed his suggestion.

Fatty Acids. Stebnitz and Sommer⁽²⁶⁾ and Nelson and Dahle⁽⁵⁾ thought that the development of this flavor was caused by the oxidation of the double bonds in certain fatty acids. The latter⁽⁵⁾ did further work on this. They homogenized a phospholipid free fat into milk and found that it developed the typical oxidized flavor. Thus they felt justified in claiming that it was the fat and not the phospholipids that caused oxidized flavor in milk.

Phospholipids. Perhaps the most recent and thorough work along this line is by Swanson and Sommer⁽⁴⁹⁾ who attempted to determine whether the phospholipids or the fat was responsible for the flavor. By using a complex ether extraction method they were able to separate the fats from the phospholipids. A large sample of milk was pasteurized and divided into two parts. One was allowed to set three days to develop an oxidized flavor, and then it was churned to obtain the fat and phospholipids. The other sample was churned at once. The iodine numbers of the fats and phospholipids in each case were found and compared.

No appreciable difference was noted in the iodine number of the fats, but that of the phospholipids was much lower from the milk that was allowed to develop an oxidized flavor. This experiment was re-

peated using cream instead of milk, and identical results were obtained.

Because the change in the iodine number of the phospholipids coincided with the development of oxidized flavor, they concluded that the phospholipids were responsible for the development of this flavor.

Another point of interest was that the purified phospholipids changed from their usual white color to a gray, indicating a chemical change.

It has been suggested that sunlight will have the same effect on the phospholipids in the milk and develop the "sunshine" flavor which is considered the same as oxidized.

On the surface, it seems that various authors^{(5) (26) (49)} are not in agreement as to the cause of the flavor. However, each of these authors may be partly right. It may be possible that the first noticeable oxidized flavor is due to the change in the phospholipids, and this flavor later intensified by an oxidation of the double bonds of the unsaturated fatty acids present.

PREVENTION

Copper. Progress has also been made along the lines of preventing the development of oxidized flavor in milk even though the exact cause is not known. It has long been known that a minimum of this copper and iron contamination will do much to curb the flavor. Keeping the milk free from this metallic contamination is mainly a problem of the milk plant operator. He should keep surfaces of equipment, which come into contact with the milk, free from exposed copper. With the use of more of the alloys mentioned by Henderson and Roadhouse⁽¹⁹⁾ this will be much easier to do.

Feeding. The wise feeding of dairy cattle also seems to be of value in preventing the development of oxidized flavor. Several workers (24)(38)(39)(27)(21) have fed both vitamins A and C with very good results. Although Henderson⁽⁵⁰⁾ did report that carotene seemed to help the flavor, he did not recommend that it be used. He gave no reason for such a statement, but perhaps it was because he was not sure that it would be the best practice in all cases.

There is little that the milk plant operator can do about the feed that the cow receives. He has to find other means of preventing the development of oxidized flavor in the milk. Martin⁽⁷⁾ mentions several possible means of controlling this flavor. In addition to recommending a minimum of exposed copper he suggested that (1) only milk of normal composition, especially in respect to carotene content, and of low apparent acidity, be used, (2) milk should be protected from sunlight and freezing, and (3) a short storage period be used for all dairy products.

Removal of Oxygen. Guthrie, Hand, and Sharp⁽³⁴⁾ suggested that the removal of oxygen from the milk would prevent the oxidation from occurring. Dahle⁽⁵⁴⁾ found this to help to some extent, but it did not eliminate the trouble. In addition he recommended the use of heat, culture, homogenization, and antioxidants. He found that hydroquinone, maleic acid, and vitamin C were the best antioxidants studied. He did not secure very good results with the direct addition of carotene.

Antioxidants. Dahle and Josephson⁽⁵¹⁾ tell of successfully using oat flour to prevent the oxidized flavor in butter. They indicated that this might also be useful in milk. Later they reported⁽⁵²⁾ that use of

paper milk bottles lined with oat flour did much to prevent the oxidized flavor from developing, and that this method was more desirable than adding the flour to the milk itself. Garrett⁽⁵³⁾ obtained similar results using paper bottles treated with the oat flour.

Amino Acids. Corbett and Tracy⁽⁵⁵⁾ had reason to believe that the proteins in the milk might be the factor that acted as an antibody to prevent the oxidized flavor from occurring. They added several amino acids to milk that would ordinarily become oxidized and noted their effect. Most of those added gave objectionable flavors. However, tyrosine and tyrosine ethyl ester did not affect the flavor and prevent the occurrence of oxidized flavor even when added at the rate of .02 to .03 per cent.

Enzymes. Although Anderson⁽⁵⁶⁾ is the only author reporting the use of pancreatic enzyme, his results are so conclusive they should be considered. This enzyme eliminated the occurrence of oxidized flavor in the plants where it was used as well as in the laboratory. He recommended the addition of the enzyme in such concentrations necessary to prevent the flavor. Usually he found that 12.5 p.p.m. was sufficient and that more than 25 p.p.m. was never required. The enzyme was added to the milk before pasteurizing so that it was destroyed on pasteurization and could not develop other off flavors. He also warned against leaving the enzyme in warm milk as this would cause the development of other off flavors.

Although the literature cited seems to indicate that the problem in general is quite well in hand, there are several phases of the problem

that are yet to be solved. Ruehe⁽⁵⁷⁾ mentioned a few of these that need to be cleared up before there will be a thorough understanding of the cause and prevention of oxidized flavor. In the first place cows on the same ration give different milk. Then, too, cows that are fed the same throughout the year still show difference due to the seasons. He also questioned the effect that bacteria and weather have on the milk.

RECENT LITERATURE

A review of oxidation flavors in milk by Brown and Thurston⁽⁵⁸⁾ was published too late to be included in the review of literature in the appropriate place, but since it is perhaps the most recent and the most inclusive of all the literature available, it should be mentioned. However, they reported no new findings in the field, but rather reviewed what others have found. They went into great detail on certain points, but nothing that has not already been discussed was brought up.

PART II

ORIGINAL INVESTIGATION

PROCEDURE

Much research work has been conducted on dairy products, but little of this work has been done in the South. This climate results in different feeding practices and herd care, producing milk that is different in many respects from that found elsewhere. For this reason some original investigations should be carried out in the South to meet the problems of the dairy industry of this section. Since milk is the basic product from which all other dairy products are made, investigations should first be made concerning it.

This project, started in the fall of 1939, is part of a research program to study the quality and composition of milk and other dairy products at the University of Tennessee. In this field of investigation it seemed to the author that the flavor of milk was the most important problem confronting the industry at the present time, and of the off flavors present in milk, oxidized flavor seemed to be the most objectionable. Accordingly, the following procedure was planned in order to make a study of oxidized flavor in milk.

Occurrence. Samples of milk from the local milk plants, from the herds furnishing milk to the University Creamery, and from the individual cows of the University herd were examined to note the occurrence of oxidized flavor. In this survey it was also hoped that some indication as to the cause of the flavor might be evident.

The quality of the milk of different local plants was judged by pint samples collected once a week during the winter of 1939-40. An attempt was made to secure the samples in such a manner that the milk would be comparable to that which reached the consumer. The routeman of

the creamery was instructed to pick up pints of milk from the trucks of the other plants as he met them on the streets. He kept this milk in his truck until he returned from the route, at which time he placed the samples in the milk storage room at 40 to 45 degrees F. The milk remained here until the following morning, at which time it was warmed to 80 degrees and scored for flavor. The milk was scored organoleptically by two instructors of the Dairy Department. At first the opinions of both were taken, but their scores checked so well it was necessary for only one to judge the samples. Occasionally both would examine the milk at the same time to be sure that they would still agree. Special attention was paid to the occurrence of oxidized flavors.

Several different dairy farms sold milk to the University Creamery for market milk and cheese. Each week half pint samples were taken from the milk from each of these farms. As each shipper's milk was dumped into the vat and weighed, the sample was taken. In this manner it was possible to get a composite from each herd that furnished the Creamery with raw milk. In case all the milk could not be dumped at one time, two samples were taken. After all the samples had been taken they were cooled in the milk storage room to 45-50 degrees F.

At this time, usually about two hours after the samples were taken, they were warmed to 80 degrees F. and scored for flavor. The remaining milk was then pasteurized in the bottles at 142 degrees F. for thirty minutes. The milk was heated to the pasteurization temperature in about ten minutes by placing the bottles in a sink filled with hot water. When the milk in the bottles reached 142 degrees F. cold water was added to that in the sink to bring the temperature to 142 degrees F. so that the

milk would remain at this temperature. A circulating pump turned by a small electric motor kept the temperature of the water bath the same at all points, while the milk in the bottles was agitated by shaking them by hand every five minutes.

At the end of the pasteurization period the sink was filled with cold water and the samples of milk cooled to below room temperature. In the summer it was necessary to add ice to the water to accomplish this. After the milk had cooled, to allow some of the cooked flavor to escape it was again warmed to 80 degrees F. and scored for flavor.

During all this processing the milk did not come in contact with enough exposed copper to induce the development of oxidized flavor. For that reason it was necessary to add copper in the form of copper sulfate at the rate of 2.5 to 3.0 p.p.m. A solution of one per cent copper sulfate was added at the rate of .25 ml. per 200 ml. of milk. The milk was then placed in the cold storage room for twenty-four hours, warmed, and judged again. This time only the amounts of oxidized flavor present were indicated. A score of zero was given if no oxidized flavor could be detected. One plus mark was given for milk that was slightly oxidized and two marks were given if the flavor was definitely noticeable. Three plus marks were given to all milk that was strongly oxidized while four plus marks indicated that the milk had become so oxidized that it was unfit for human consumption.

The milk was again cooled and stored for an additional forty-eight hours, when it was warmed and judged again for oxidized flavor. The milk was scored as described in the above paragraph. During the first part of the experiment the milk was also judged for oxidized flavor on the fifth

day. However, because other flavors developed, and because the results of the first and third days clearly indicated the degree of susceptibility of the milk to become oxidized, this was discontinued.

A study of the milk of the individual cows of the University was conducted along the same lines. The only difference being the method of collecting the samples. At the time of milking, samples of milk were collected in half pint bottles. The milk was taken directly from the pail or milking machine immediately after each cow was milked. Within two hours after the first cow was milked, the samples were taken to the cold storage room to cool. After this they were treated exactly the same as those samples taken at the creamery.

One set of samples from individual cows was secured from one of the dairy farms where the milk usually became oxidized. The samples were taken in the same manner as those taken from the University herd and received the same treatment.

Feeding practices, lactation periods, and age of the cows were secured from the records kept on the University herd in an attempt to find some correlation to the occurrence of the oxidized flavor.

Prevention. In an attempt to find a means whereby the occurrence of oxidized flavor could be prevented, the effects of certain antioxidants were studied. Limited facilities made it possible to study only two of these and after some review of the literature, it was decided to use vitamin C and Avenex, which is an oat flour extract. Preliminary trials indicated that the Avenex could not be used in this study as off flavors resulted when enough of it was added to prevent the occurrence of oxi-

dized flavor. Had it been possible to process the milk in a different manner this difficulty might have been eliminated. However, because of this, sodium thiosulfate was used instead.

By the time this work was started, the investigations on the milk from the shippers to the University Creamery and from the cows of the University herd had progressed far enough so that it was possible to secure milk that was known to develop an oxidized flavor. Two-quart samples of milk were pasteurized at 142 degrees for thirty minutes in a two-liter beaker. The beaker was heated by a spray of hot water running down its sides and the milk was agitated by a glass stirring rod connected to an electric motor. The milk was cooled with cold water in a similar manner. After the milk had cooled, it was placed in eight half pint bottles and treated as follows:

One of these was used as a control and nothing was added.

Copper was added to the seven other bottles at the rate of 2.5 p.p.m. To six of these, antioxidants were added, but the seventh received no further treatment so that a check could be made to see that the oxidized flavor would develop in the milk. Half of the remaining samples were treated with the vitamin C in the form of tablets. Approximately 6 mgm., 12 mgm., and 18 mgm. were added per 100 ml. of milk.

The sodium thiosulfate was added to the other three at the rate of $12\frac{1}{2}$ mgm., 25 mgm., and 50 mgm. per 100 ml. of milk. Because the $12\frac{1}{2}$ mgm. seemed to have no effect, 100 mgm. of the thiosulfate was added to this sample instead.

These samples were then stored at 40-45 degrees F. for twenty-four hours. At the end of this time they were examined organoleptically

for oxidized flavor as were the samples of the individual herds and cows for the presence of oxidized flavor. The milk was again placed in storage and scored on the third and fifth days after pasteurization. Although the milk was not scored on other flavors, if a noticeable flavor developed that might have been due to the antioxidants, mention was made of it on the score card.

RESULTS

Market Milk. In the survey of the milk from the plants of Knoxville, Tennessee, during the winter of 1939-40, one hundred and fourteen samples were scored. Of this number the scores of only ninety-one are listed as the remaining twenty-three represent samples from fourteen different distributors and it is probable that a good indication of the milk of these plants cannot be obtained from these limited data. The ninety-one samples used represent the milk of eleven different distributors in the city.

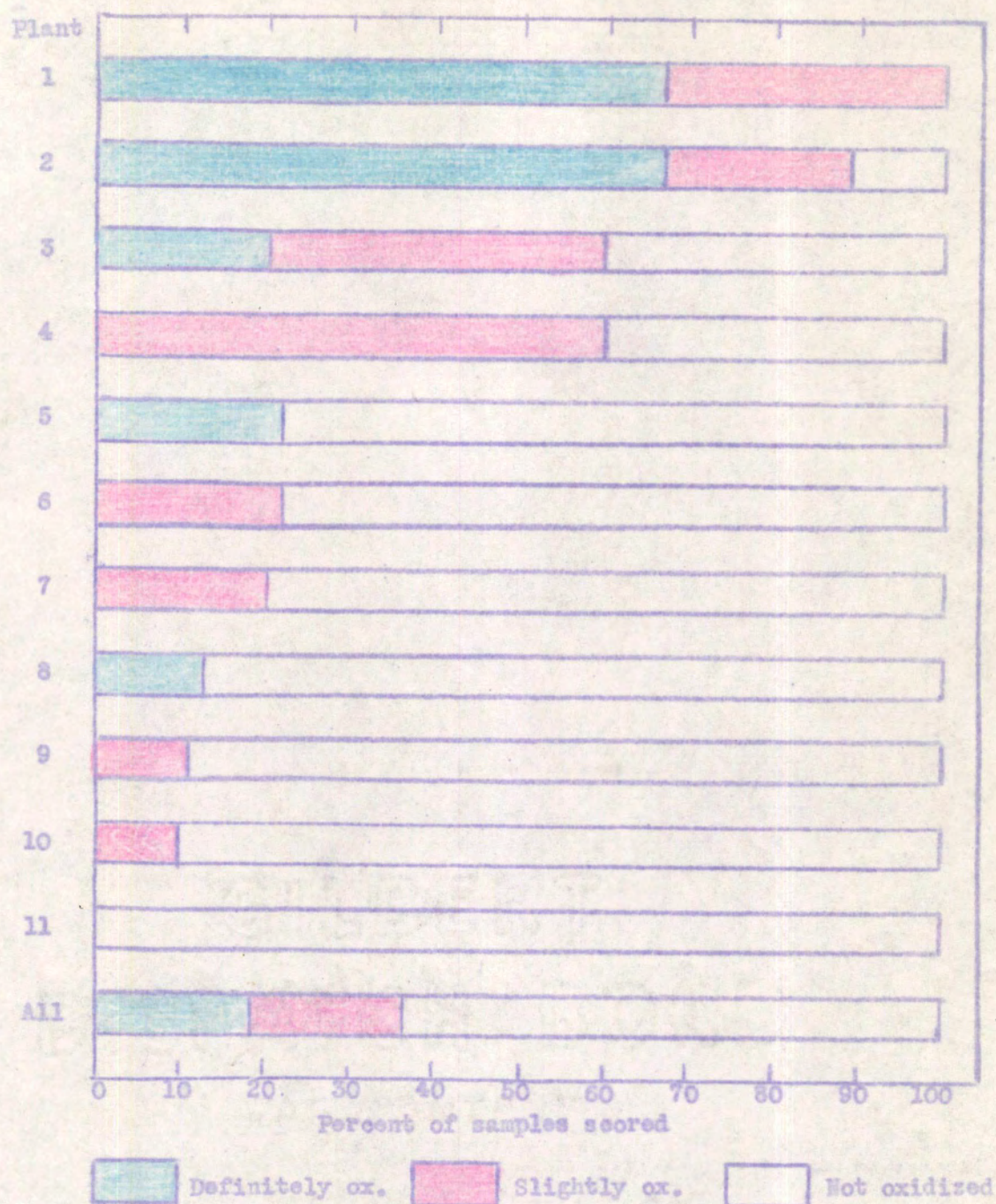
Table I lists the occurrence of the oxidized flavor in the milk from the different plants. Of the ninety-one samples, 18.7% showed signs of a slight oxidized flavor that was not serious and would not likely be noticeable to the consumer. However, it is very probable that the concentration of the flavor in these samples would have increased as time went on. In addition, there were 18.7% that indicated a definite oxidation that would make the milk objectionable to the consumer. In all, there would then be about thirty-eight per cent of the milk that was criticized as oxidized. This is higher than found by Martin⁽⁷⁾ who reported from ten to twenty per cent of the market milk he scored was criticized as being oxidized. This difference tends to point out that the

TABLE I. OCCURRENCE OF OXIDIZED FLAVOR IN MILK FROM MILK PLANTS IN KNOXVILLE IN THE WINTER OF 1939-40

Plant	Samples scored	Slightly oxidized		Definitely oxidized		Total oxidized	
		Number	Per cent	Number	Per cent	Number	Per cent
1	9	3	33	6	67	9	100
2	9	2	22	6	67	8	89
3	10	4	40	2	20	6	60
4	5	3	60	0	0	3	60
5	9	0	0	2	22	2	22
6	9	2	22	0	0	2	22
7	5	1	20	0	0	1	20
8	8	0	0	1	12½	1	12½
9	9	1	11	0	0	1	11
10	10	1	10	0	0	1	10
11	8	0	0	0	0	0	0
Total	91	17	18.7	17	18.7	34	37.4

problem is not the same in all localities.

When the values listed on Table I are put in graphic form (Figure 1) it is easy to see that four plants were responsible for most of the oxidized flavor. Although only thirty-seven per cent of the samples were taken from these four plants, they were responsible for over 76% of the oxidized flavor. Three of these plants, one, two, and three, representing about thirty per cent of the samples, were responsible for eighty-two per cent of these samples that were oxidized to the extent that they would be objectionable to the consumer.



Source: Table I

Figure 1. Occurrence of oxidized flavor in market milk from plants in Knoxville, Tenn. November to February, 1940

Although it was possible to inspect only four of these plants, the conditions of these plants partially indicated the degree the milk would become oxidized. Plant two is a small dairy operated some distance from the city, processing milk from nearby farms. The plant had recently been remodeled and the first impression is that there would be little danger of the milk developing an oxidized flavor. However, close inspection revealed that most of the tinning from the sanitary pipe lines was worn off, leaving much exposed copper to come in contact with the milk. Plant four is a small plant that specializes in producing milk of high fat content and of low bacterial count. There is not any great amount of copper exposed to the milk and there is no apparent reason for the development of the oxidized flavors in this milk. This is not unusual, however, as Roland et al.⁽⁶⁾ reported that oxidized milk occurs more often in milk of high fat content and low bacterial count.

Plant six is the University Creamery where an attempt is made to keep the copper contamination in milk to a minimum. This is reflected in the small percentage of milk from this plant that was oxidized. Only two of the nine samples taken had any oxidized flavor present, and of these two, neither was criticized as being objectionable to the consumer.

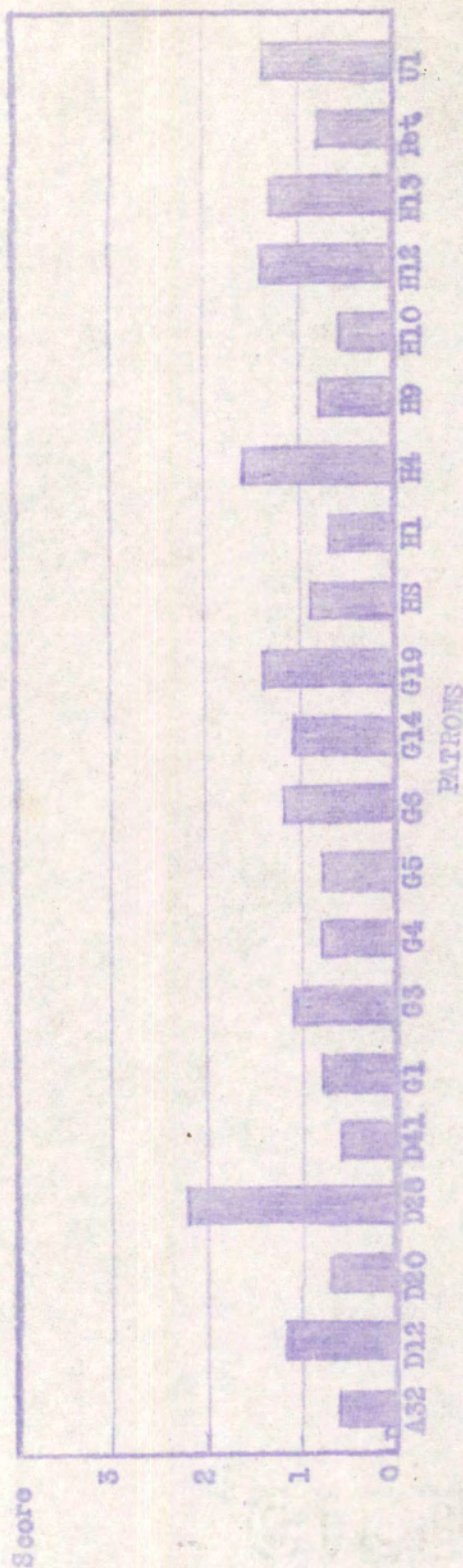
Samples of milk from plant ten indicated that this milk was less likely to become oxidized than that from the other three. This plant is one of the largest in the city, distributing more milk than the other three plants combined. Care was taken to insure a minimum of copper contamination which is about all that any plant does at the present time to check the oxidized flavor. The milk for this plant is bought from farmers

while the other three get a large part of their supply from their own herds. For that reason it might be that the milk delivered to plant ten has a higher bacterial count, another factor thought to be associated with the prevention of oxidized flavor. However, it cannot be definitely stated that this is the reason plant ten had less oxidized milk.

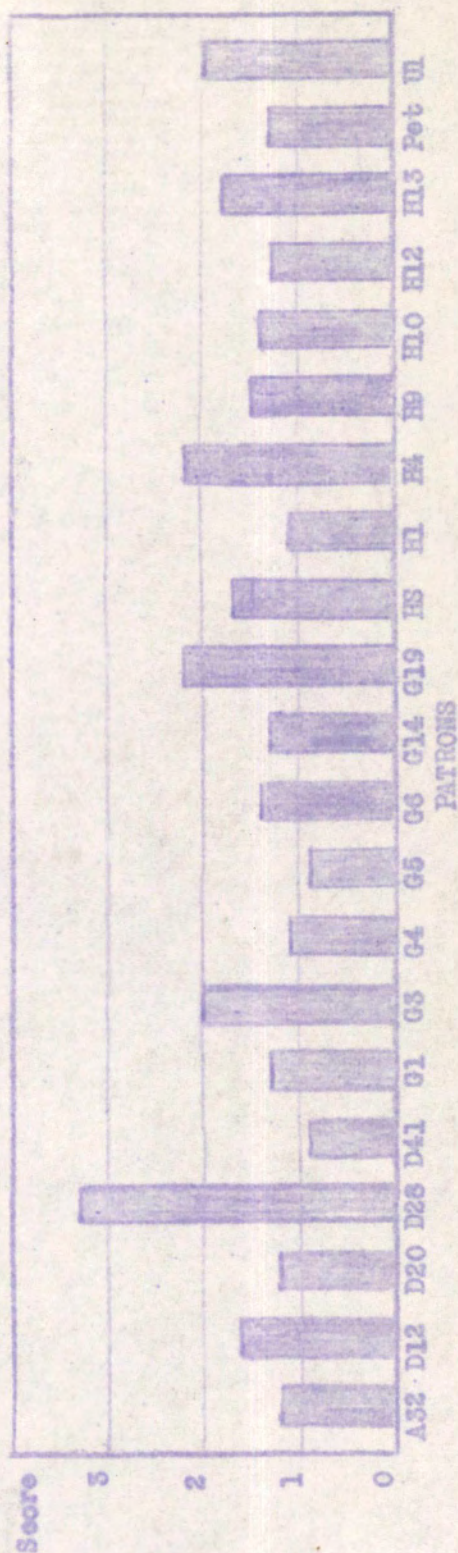
Herd Composites. Over four hundred samples were taken from the milk received by the University Creamery during the period of January to July, 1940. In cases where more than ten samples were obtained from one herd, the average oxidized score for the first and third days was calculated and is recorded in Table II. Graphs (Figures 2 and 3) of the first and third days' oxidized flavor score show more clearly the variance in

TABLE II. OCCURRENCE OF OXIDIZED FLAVOR IN MILK FROM
UNIVERSITY CREAMERY PATRONS, JANUARY TO JULY, 1940

Name	Oxidized flavor--1st. day		Oxidized flavor--3rd. day	
	No. samples	Mean of scores	No. samples	Mean of scores
A32	16	.6	16	1.2
D12	21	1.2	19	1.6
D20	18	.7	15	1.2
D28	21	2.2	21	3.3
D41	14	.6	12	.9
G1	19	.8	19	1.3
G3	17	1.1	18	2.0
G4	14	.8	14	1.1
G5	15	.8	17	.9
G6	13	1.2	14	1.4
G14	17	1.1	17	1.3
G19	20	1.4	20	2.2
HS	14	.9	16	1.7
H1	19	.7	19	1.1
H4	19	1.6	19	2.2
H9	15	.8	13	1.5
H10	16	.6	16	1.4
H12	15	1.4	16	1.3
H13	13	1.3	15	1.8
Pet.	20	.8	21	1.3
U1	24	1.4	25	2.0



Source: Table II
Fig. 2. Average oxidized flavor score of milk from the patrons of the University Creamery one day after pasteurization. January to June, 1940.



Source: Table II
Fig. 3. Average oxidized flavor score of milk from the patrons of the University Creamery three days after pasteurization. January to June, 1940.

the different herds. On both graphs the milk from the herd D28 is much higher than any of the others. Although there are a few whose scores seem to be appreciably higher than the average, as with G19, H4, and U1, the difference is not so great as in the case of D28.

Investigation revealed that the herd D28 was composed of two groups of cows, one of which was on official test. To secure the maximum milk the test cows were fed all that they would eat of a ration rich in grain and beet-pulp, with good quality alfalfa hay. The other cows received a limited amount of grain and hay. The samples of the individual cows from this herd gave results which indicate that herd care is an important factor. All the cows that were on test, with the exception of one just fresh, gave milk that would become seriously oxidized when treated with copper in the usual manner. All the rest of the cows in the herd gave milk that was apparently normal with respect to the development of the oxidized flavor. Table III gives the oxidized scores for the one trial on herd D28. Since only one trial was conducted to check the milk of these cows, and since there are many variable factors, it would be impossible to reach any definite conclusions as to the cause of the flavor. Cows one to nine, inclusive, are test cows, the ninth being fresh only a few days, and the rest are cows fed on the limited grain ration. The production of milk susceptible to oxidation cannot be attributed to the low carotene intake of the test cows as they received a good hay and also had access to pasture. Some other factor that can be linked with the feed or feeding practices seems to be the cause of the cows producing milk that is susceptible to oxidation. In the study of the results from the milk

TABLE III. OCCURRENCE OF OXIDIZED FLAVOR IN MILK FROM
INDIVIDUAL COWS OF HERD D28
May 28, 1940

Cow	Oxidized score	
	1st. day	3rd. day
1	2	4
2	3	3
3	2	4
4	2	4
5	1	4
6	3	4
7	2	3
8	1	3
9	0	1
10	2	2
11	0	1
12	1	2
13	2	2
14	1	2
15	1	1
16	1	2
17	1	2
18	0	1

of individual cows of the University herd, this will be discussed further.

Individual Cows. In a study of the milk from the cows of the University herd, there were so many variable factors that it was impossible to attribute the cause of the susceptibility of milk to become oxidized to any one factor. In the six months that the herd was under observation, over five hundred samples of milk were studied. This amounted to about twenty samples from each cow that was in milk.

Figure 4, drawn from original data on file in the dairy office, illustrates the oxidized flavor score on the first and third days for every sample of milk that was taken. Each box contains the scores of one cow. Her name, age, and group classification are in the top of the

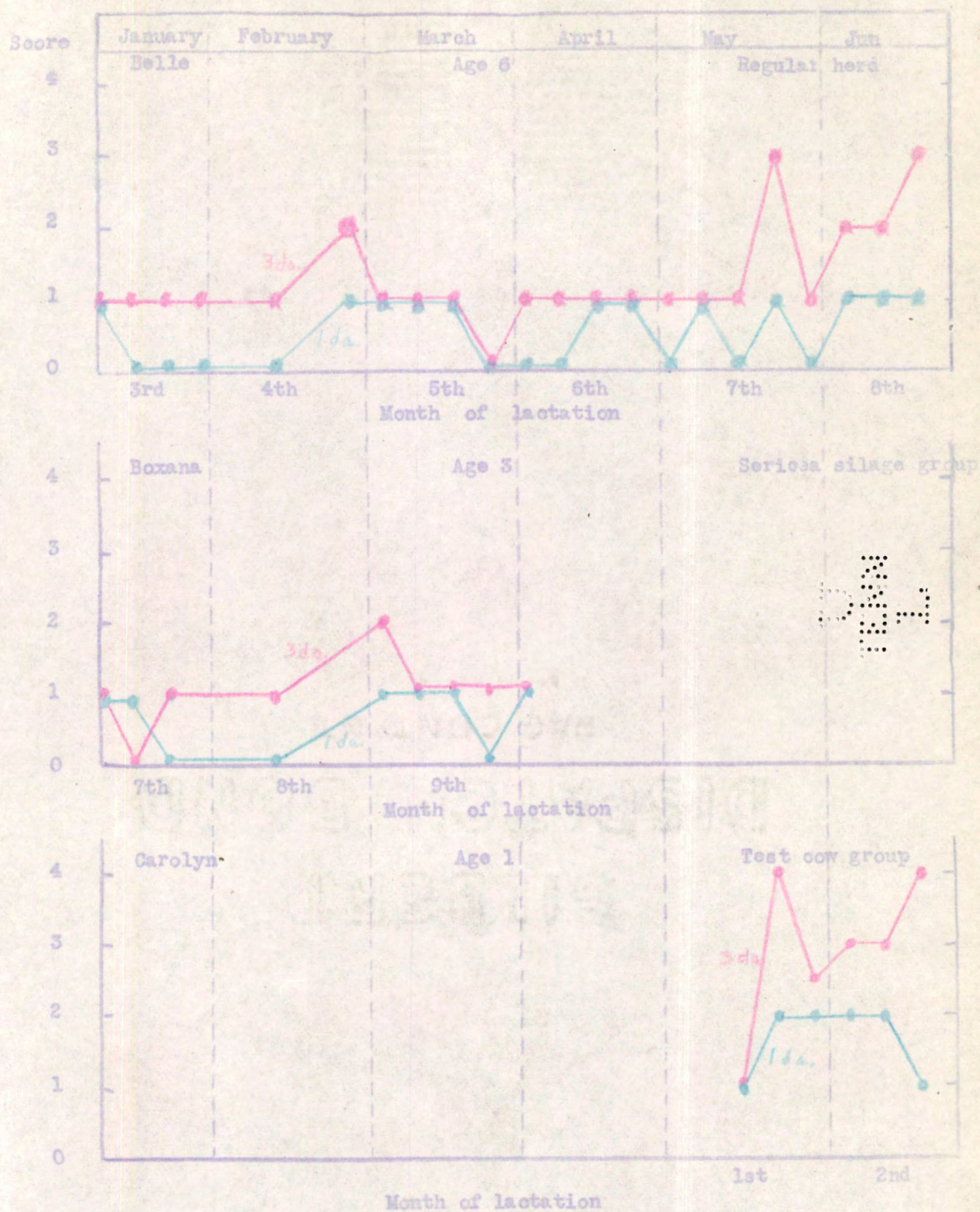


Figure 4. Occurrence of oxidized flavor in milk from individual cows of the university herd

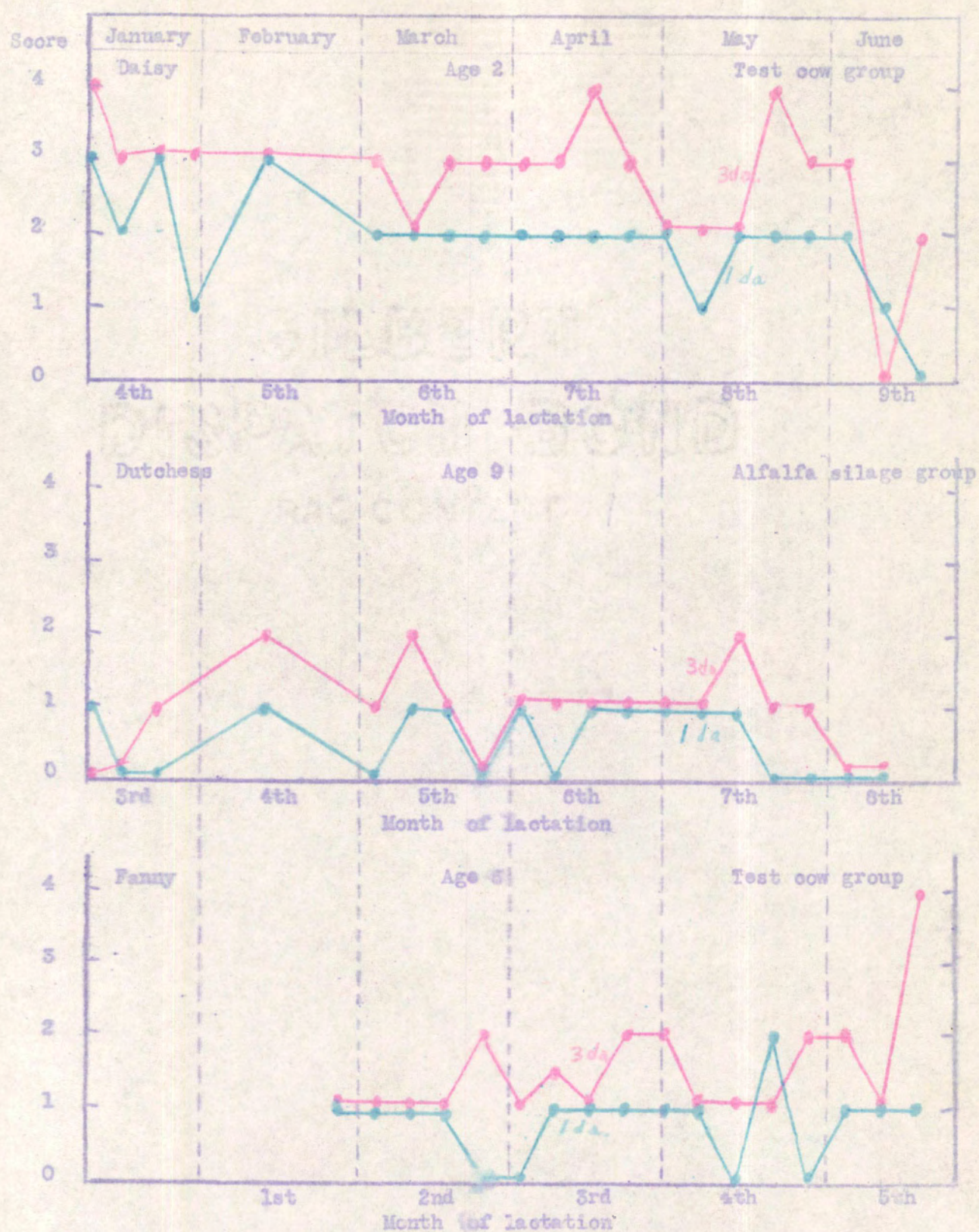


Figure 4 (cont.). Occurrence of oxidized flavor in milk from individual cows of the university herd

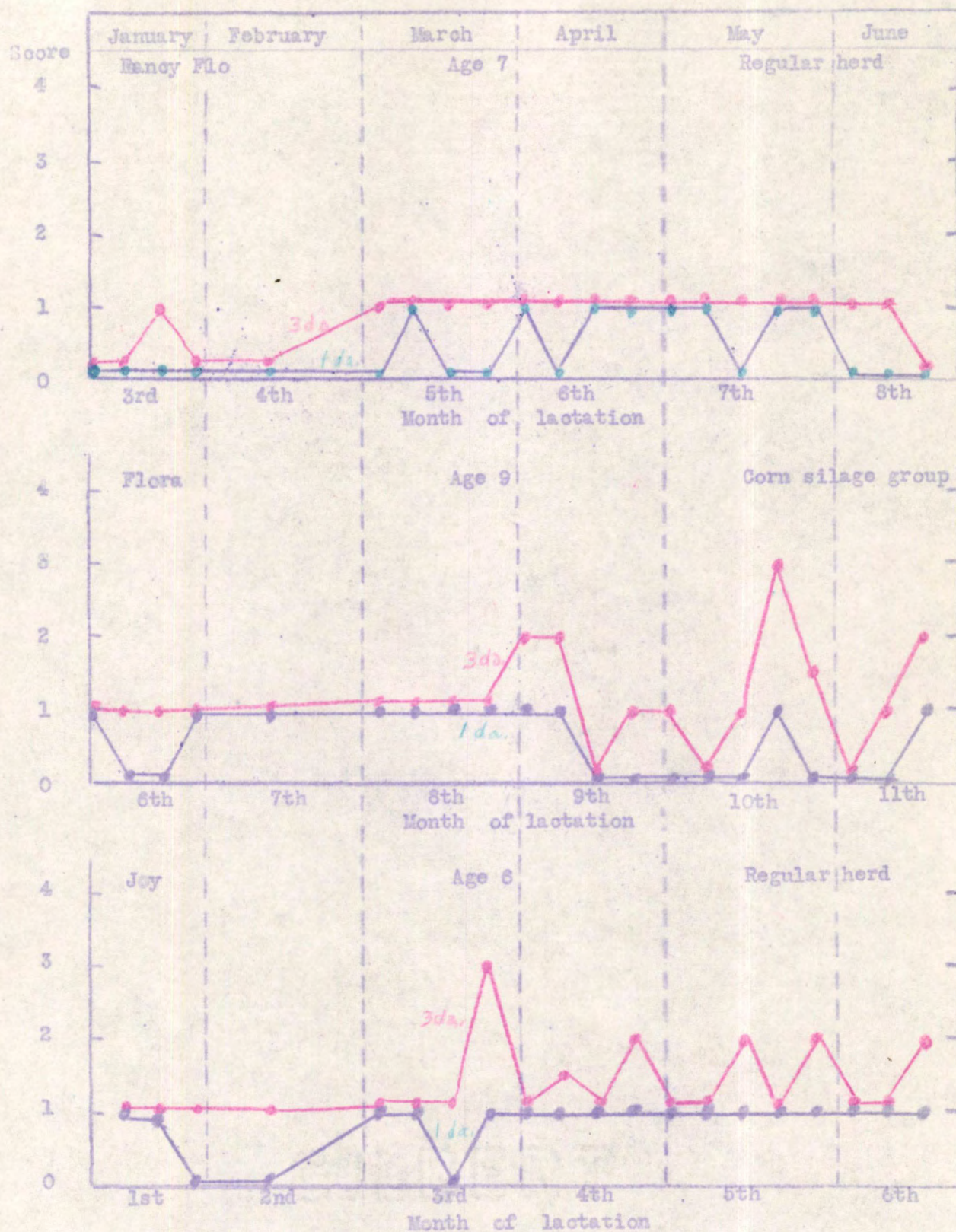


Figure 4 (cont.) Occurrence of oxidized flavor in milk from individual cows in the university herd

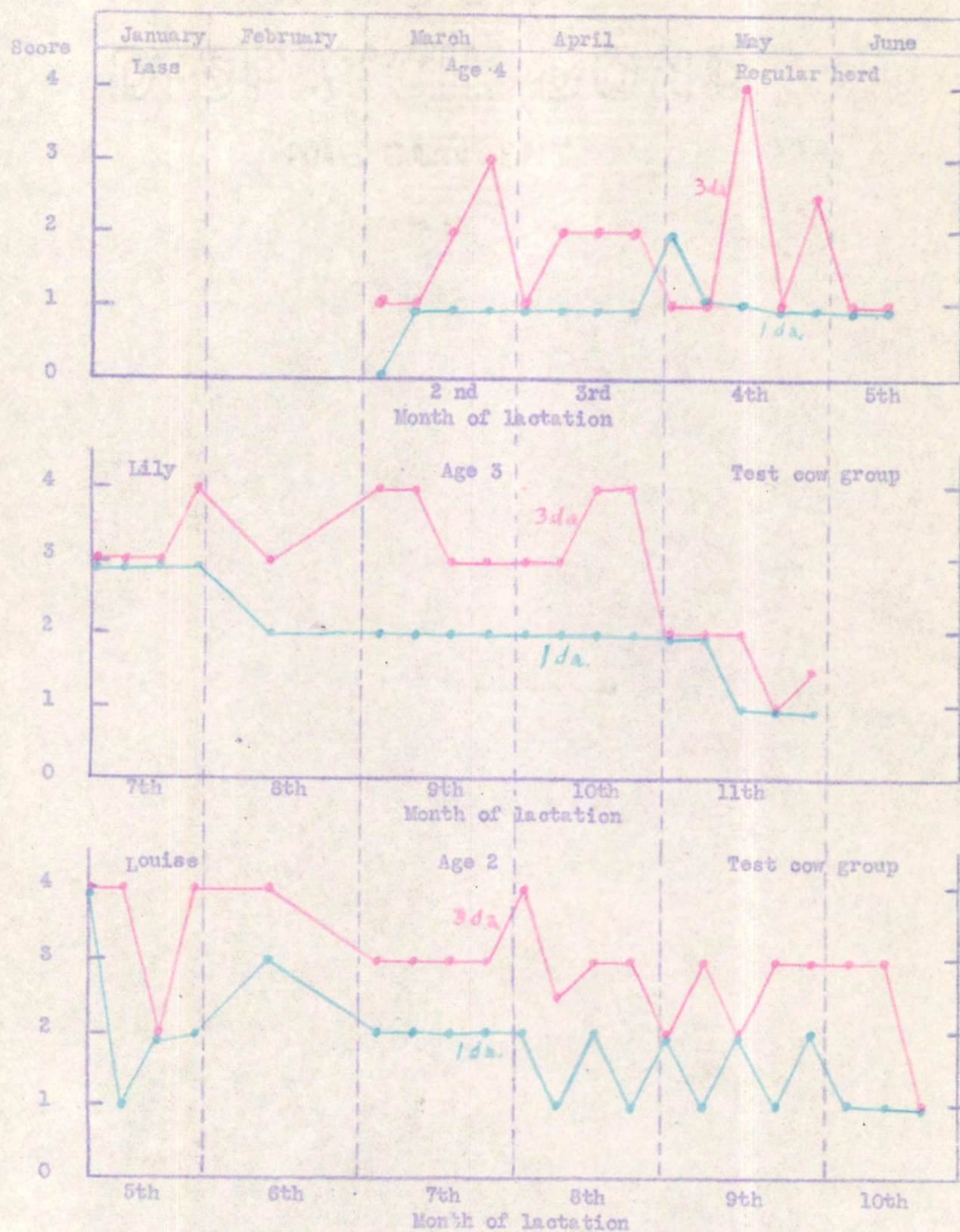


Figure 4 (cont.) Occurrence of oxidized flavor in milk from individual cows of the university herd

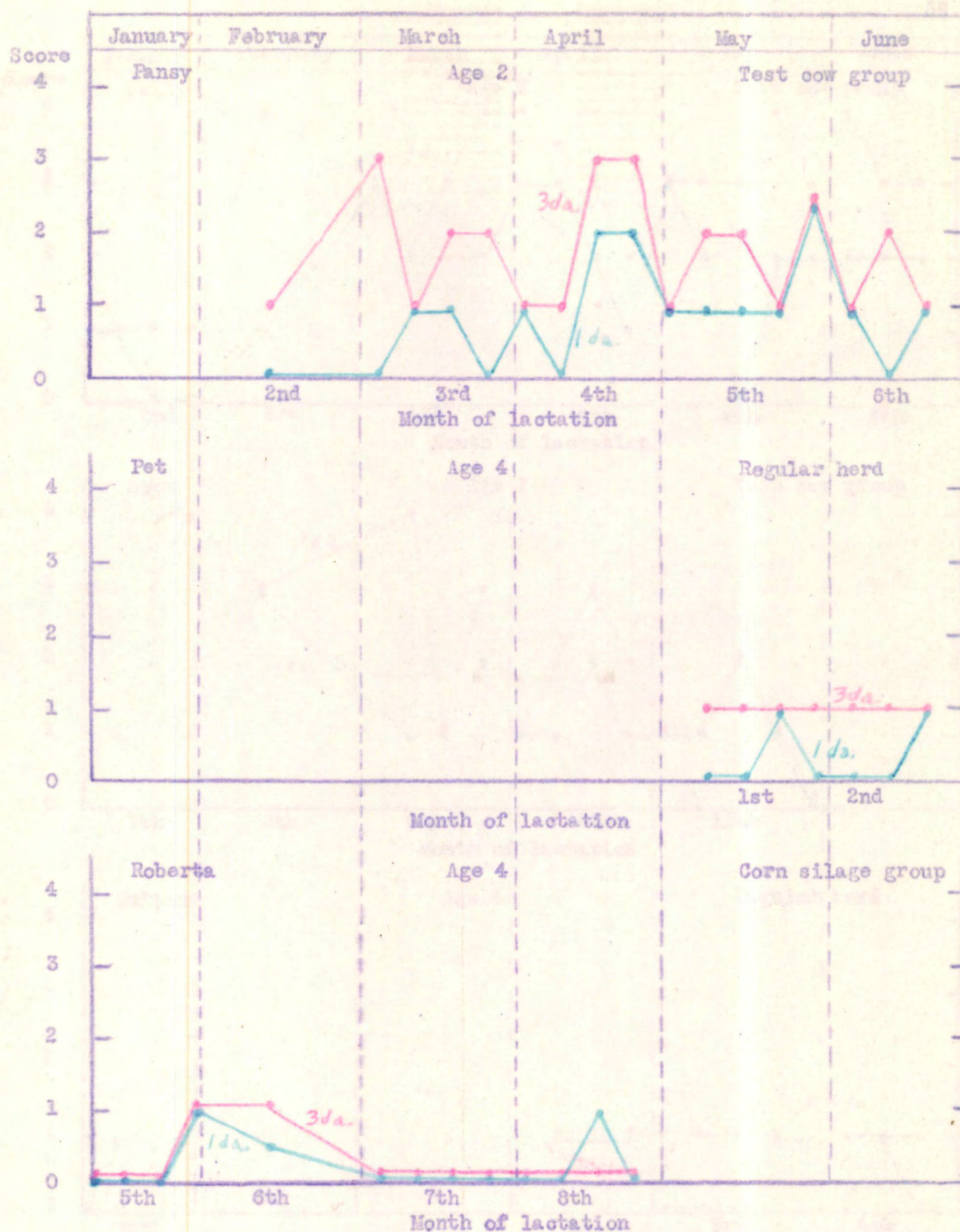


Figure 4 (cont.). Occurrence of oxidized flavor in milk from individual cows of the university herd.

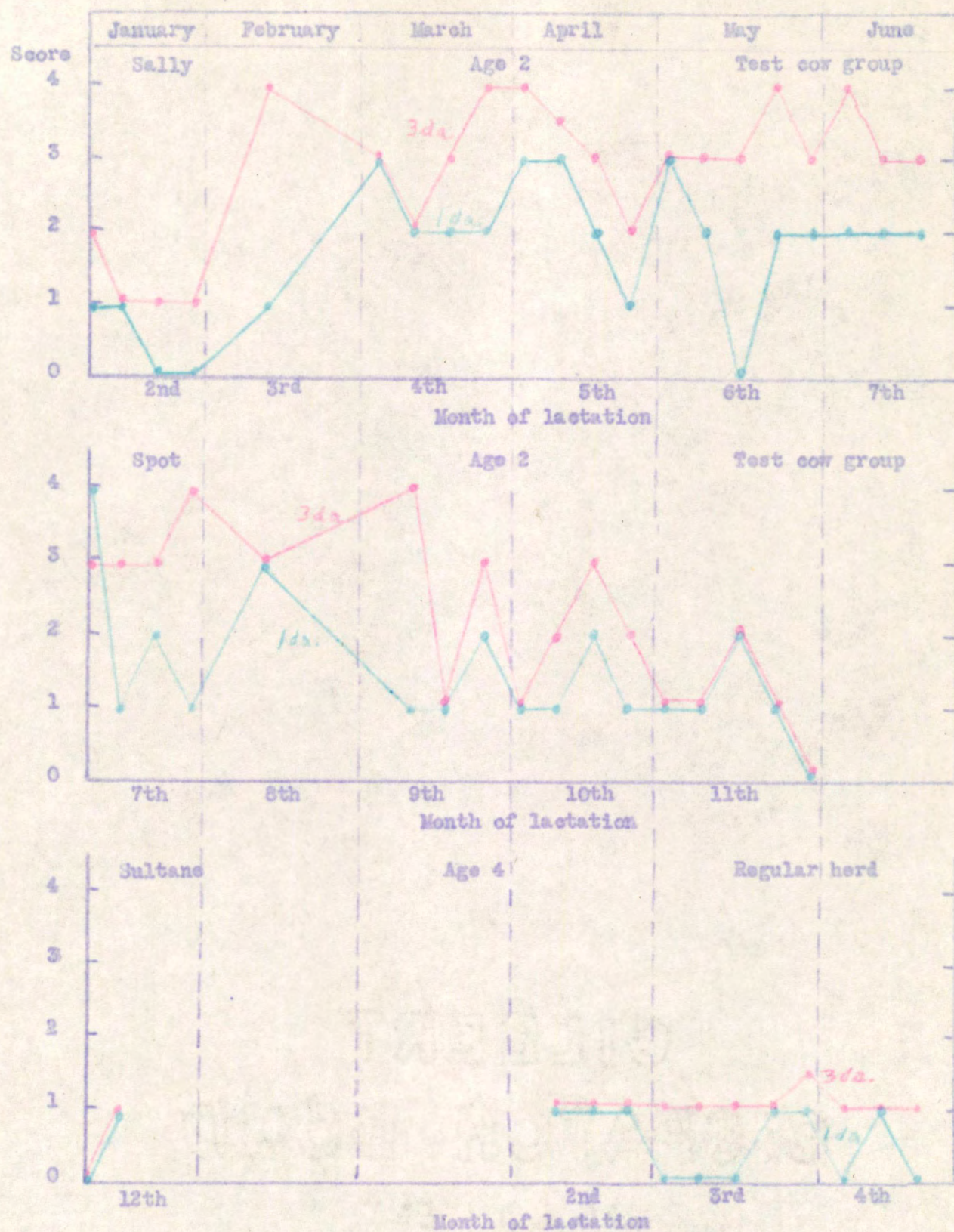


Figure 4 (cont). Occurrence of oxidized flavor in milk from individual cows of the university herd

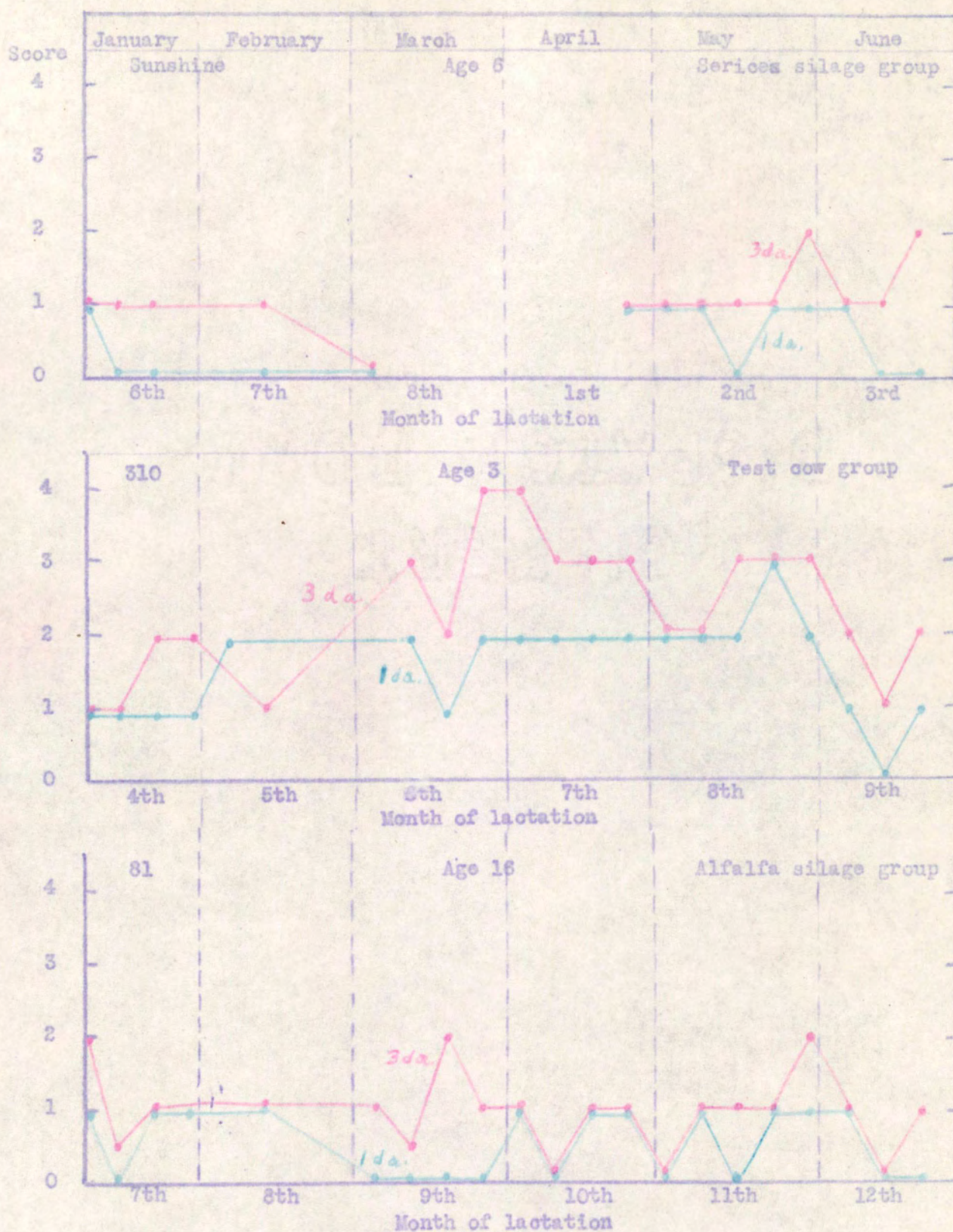


Figure 4 (cont.). Occurrence of oxidized flavor in milk from individual cows of the University herd

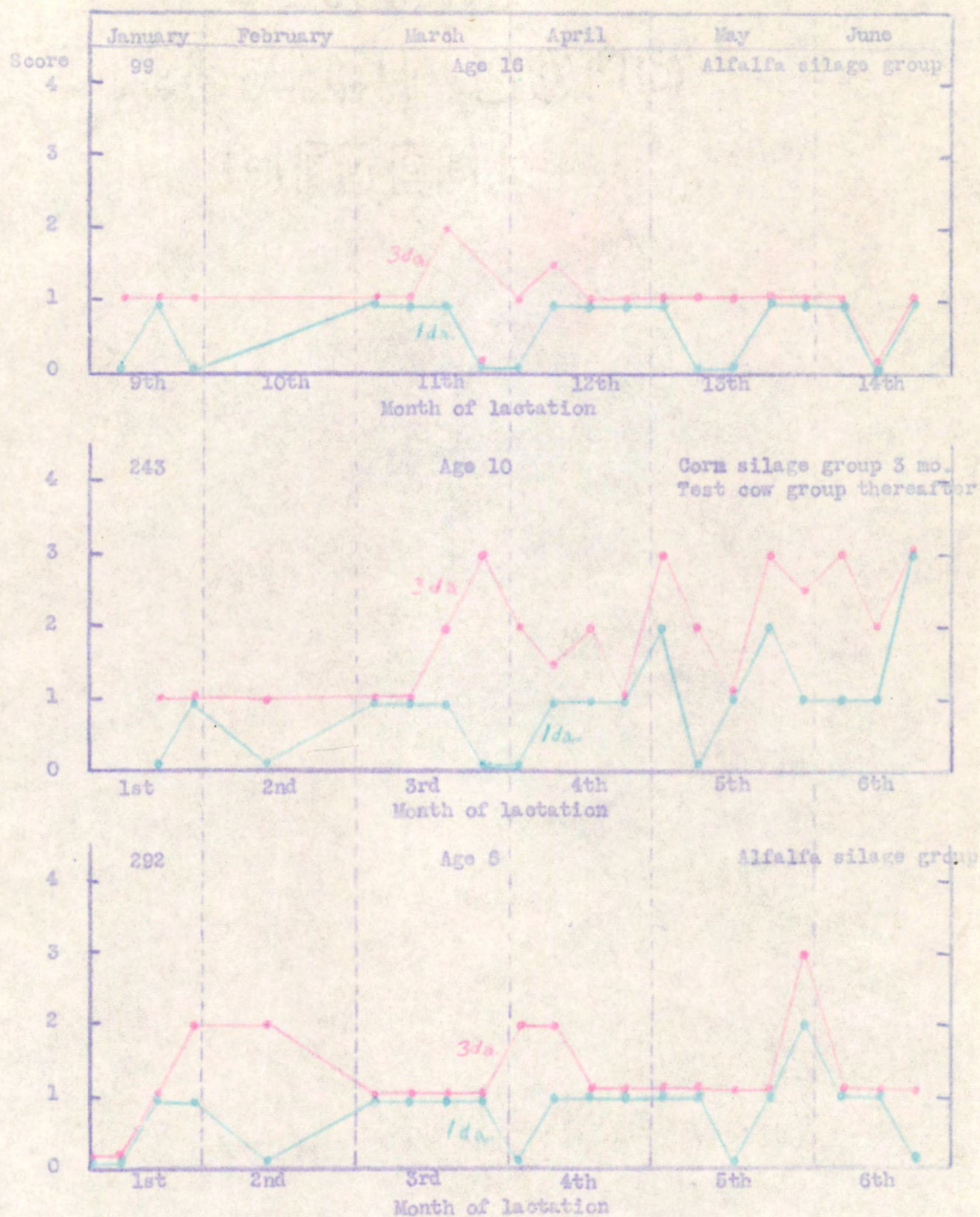


Figure 4 (cont.) Occurrence of oxidized flavor in milk from individual cows of the university herd

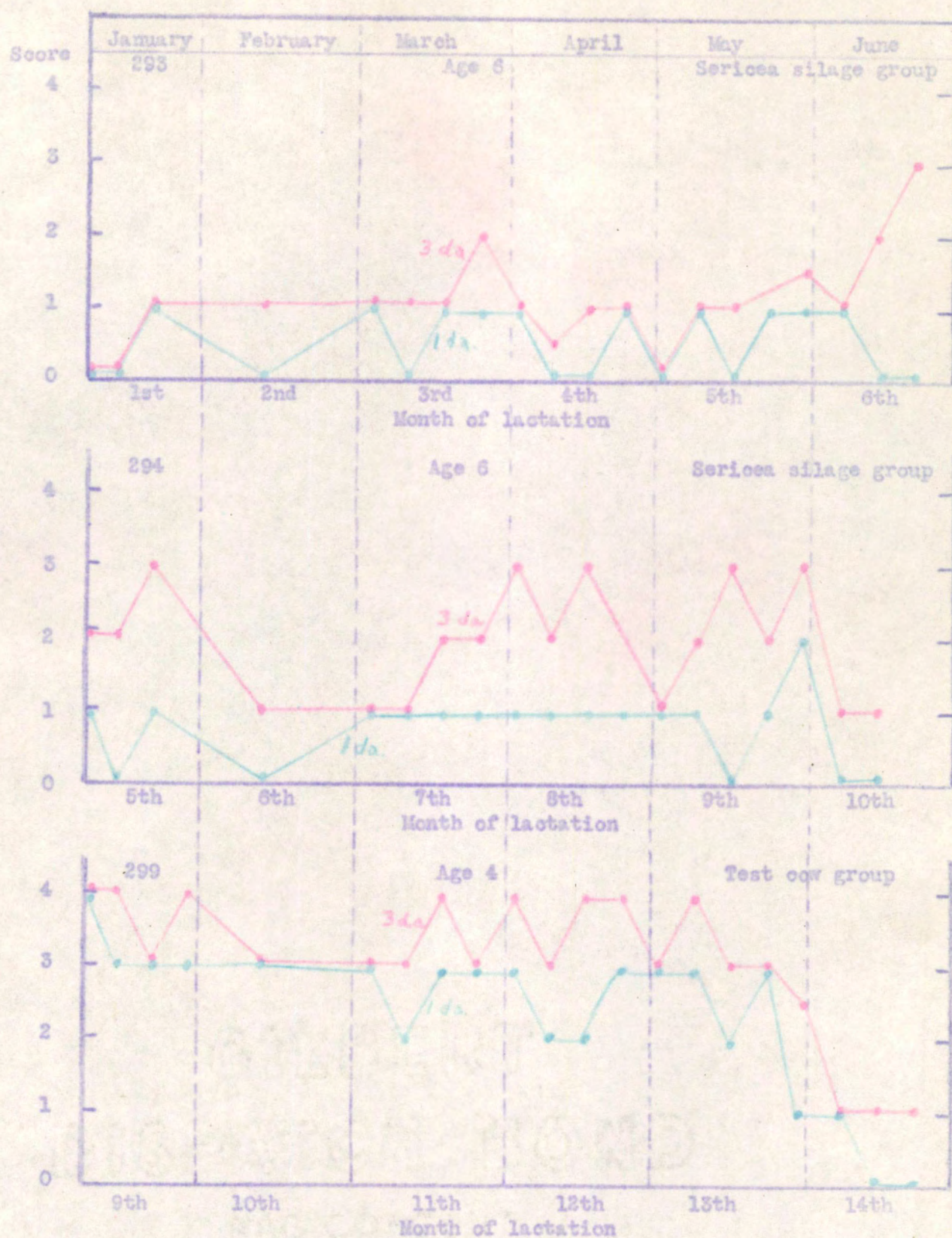


Figure 4 (cont.) Occurrence of oxidized flavor in milk from individual cows of the university herd.

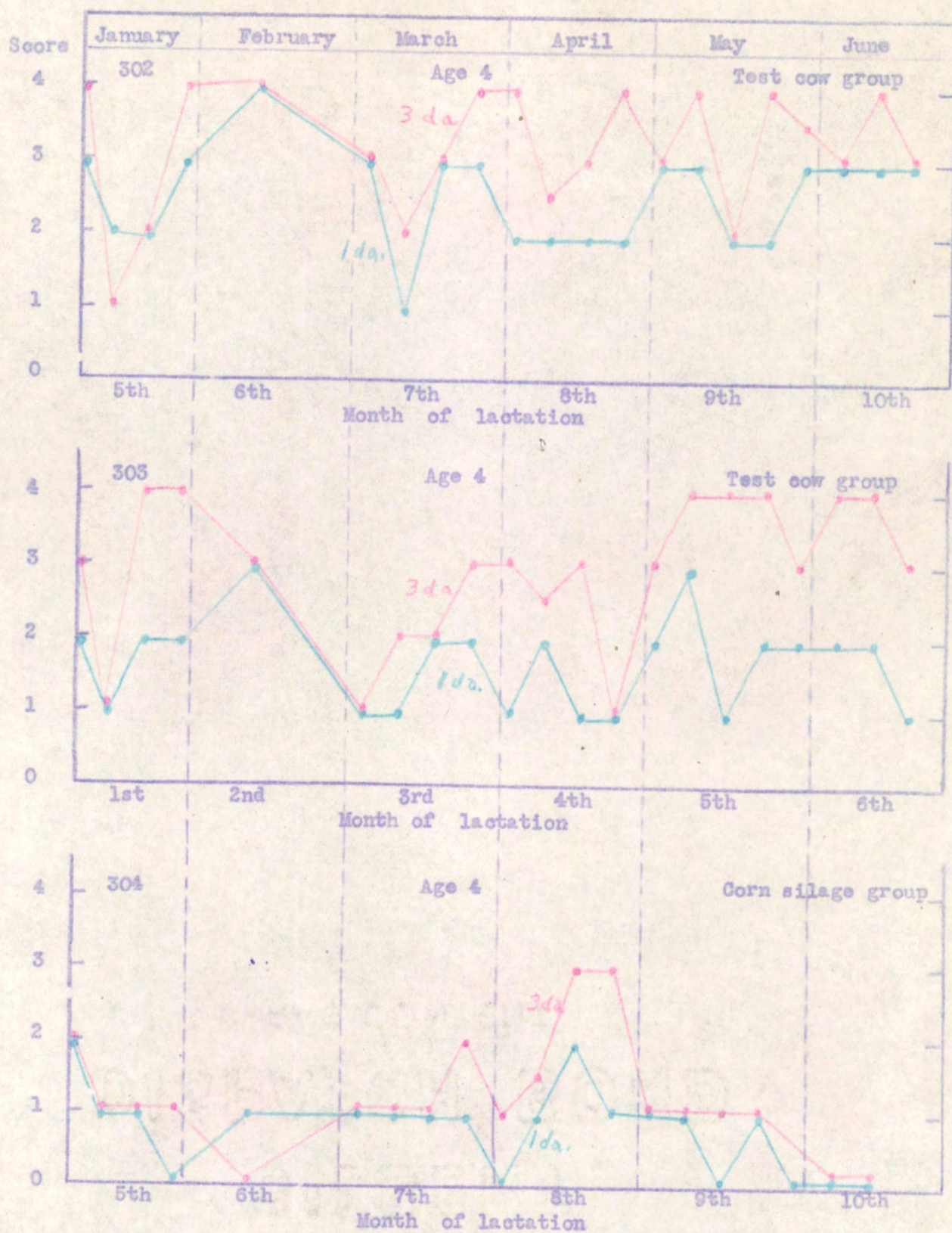


Figure 4 (cont.). Occurrence of oxidized flavor in milk from individual cows of the university herd

box. Dots indicate the date that samples were taken. These are connected by lines to give the general trend of the flavor development. Green dots and lines indicate the score for the first day, and red the third day after pasteurization.

Table IV, giving the average oxidized flavor scores for each cow

TABLE IV. AVERAGE OXIDIZED FLAVOR SCORE OF THE MILK FROM INDIVIDUAL COWS OF THE UNIVERSITY HERD, JANUARY TO JUNE, 1940

Cow	First day		Third day	
	Samples judged	Average score	Samples judged	Average score
Belle	20	.6	20	1.2
Boxiana	8	.8	9	1.0
Carolyn	6	1.7	5	3.0
Daisy	21	1.9	18	2.7
Duchess	18	.6	17	.9
Fanny	16	.8	14	1.5
Fancy Flo	19	.4	17	.8
Flora	21	.5	18	1.1
Joy	19	.9	18	1.3
Lass	15	1.0	13	1.6
Lily	18	2.0	16	3.0
Louise	21	1.8	19	3.0
Pansy	17	.9	15	1.7
Pet	7	.3	6	1.0
Robertta	12	.2	8	.3
Sally	21	1.7	18	2.8
Spot	17	1.5	15	2.5
Sultane	13	.5	10	.9
Sunshine	14	.5	12	1.1
81	18	.5	16	1.0
99	19	.6	17	.9
243a	10	1.3	9	2.2
243b	8	.5	8	1.5
292	20	.8	17	1.1
293	17	.6	15	1.1
294	18	.8	16	1.8
299a	17	2.8	16	3.3
299b	4	.5	3	1.0
302	21	2.6	19	3.2
303	21	1.7	19	2.9
304	18	.9	17	1.2
310	20	1.6	18	2.3

during the period of investigation, indicates that there is a great difference in the individual cows as to the milk they produce. While the milk from some was strongly oxidized on the first day, that from others had little of this flavor on the third day. Because two cows were changed to different rations they are listed twice. For the time that cow 299 was on test the score is listed under 299a and when she went off official test, the score is listed under 299b. In the same manner cow 243 is scored under 243a and 243b for the times she was on official test and in the corn silage group respectively. All other cows were treated the same throughout the period that samples were taken.

Figure 5, made from the results of Table IV, gives a much better indication of the susceptibility of the milk from different cows to become oxidized. It will be noted that the scores for the first and third days are closely correlated. In all cases where the average score for the first day is more than one, the average score for the third day is more than two. Likewise, those scores that are low on the first day are also low on the third day. The cow 299a has the highest average score on both the first and third days, while Roberta has the lowest average score on both days. This correlation holds true for most of the cows and both graphs appear to be much the same.

In an attempt to find if there is any possible correlation between the care of the cows and the susceptibility of their milk to become oxidized, the cows were divided into groups according to the manner in which they were fed. There were four of these groups in addition to the regular herd which was fed a limited amount of grain, hay, and a succulent

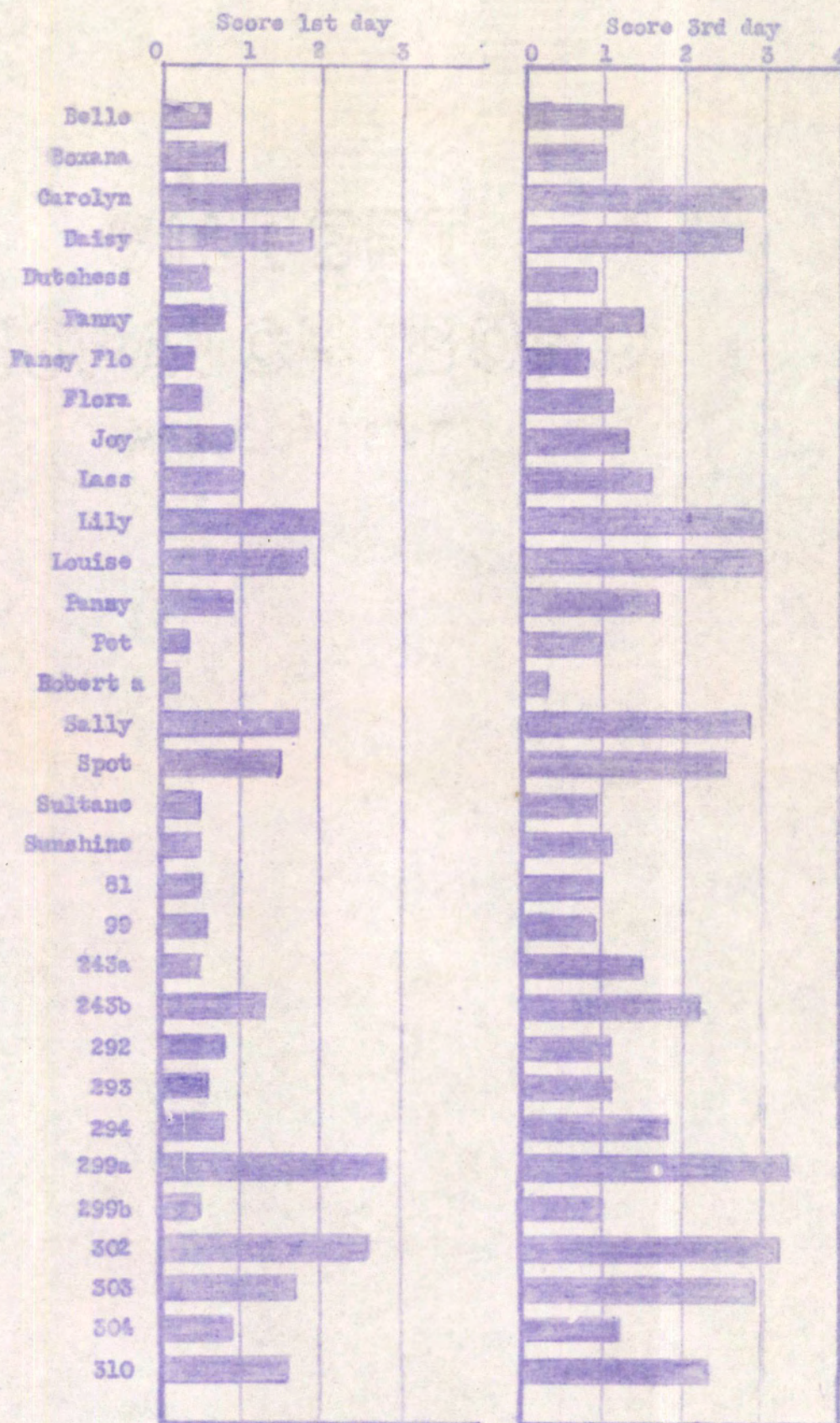


Figure 5. Average oxidized flavor score of milk from individual cows of the university herd. January to June 1940



Figure 6. Milking parlor where cows were milked

of corn silage in the winter and pasture in the summer. A group of cows on official test were fed all the grain and beet pulp mixture they could eat without injury to themselves, as well as good quality hay and succulent. During the winter an experiment was conducted to compare the value of corn, sericea, and alfalfa silages. Twelve cows were divided into three groups of four. Each of these groups was fed a different kind of silage. A grain mixture with a higher per cent protein was fed to the corn silage group to insure that all three groups would receive the same amount of total protein. The animals were fed in the open stalls where they were housed. The test cows, however, were fed the grain and beet



Figure 7. Open stalls where cows were housed and fed

pulp mixture in the milking parlor at the time of milking.

Table V gives the average oxidized score of the milk from these different groups one and three days after the milk was treated with copper. These averages are based on at least fifty samples taken during

TABLE V. AVERAGE OXIDIZED FLAVOR SCORE OF MILK FROM COWS
CLASSIFIED AS TO METHOD OF FEEDING, JANUARY TO JUNE, 1940

Group	First day		Third day	
	Number samples	Average score	Number samples	Average score
Regular herd .	87	.72	74	1.39
Test cows	226	1.62	201	2.65
Sericea silage group	60	.73	54	1.29
Alfalfa silage group	75	.63	67	.97
Corn silage group	59	.56	51	1.07

a period of six months, and for that reason it seems that a fairly accurate indication of the effects of the feeding practices should be evident. It can be seen when these data are plotted (Figure 8) that the cows which were on official test gave milk that developed a stronger oxidized flavor than the cows of the other groups. Although there is a difference in the average oxidized score of the milk from the other groups, the difference is not great enough to be considered of significance. Since the test cows receive beet pulp and molasses, as well as more grain, this might be the factor that is responsible. However, it is likely that the flavor could be due to the "speeding up" of the production of the animal by forced feeding. Cow 299 produced milk that was very susceptible to oxi-

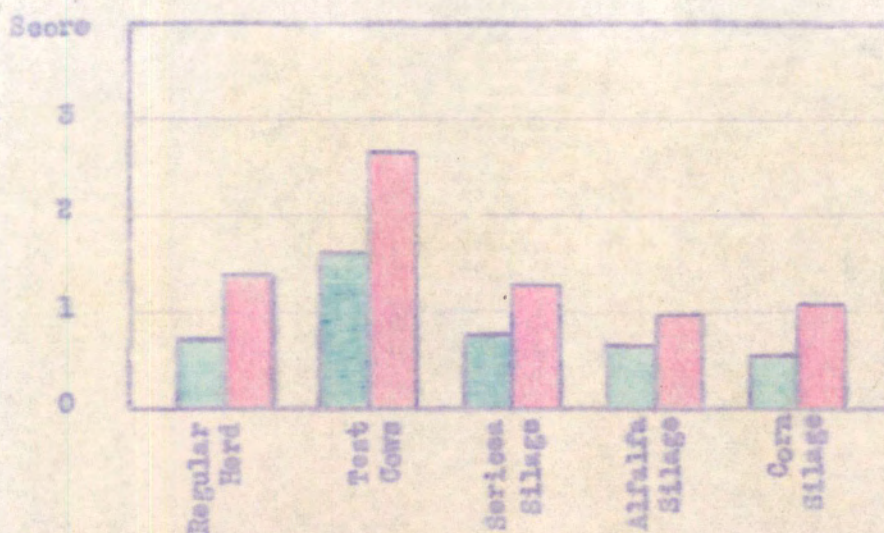


Figure 8. Average oxidized flavor score on first and third days of milk from groups classified as to feeding practices

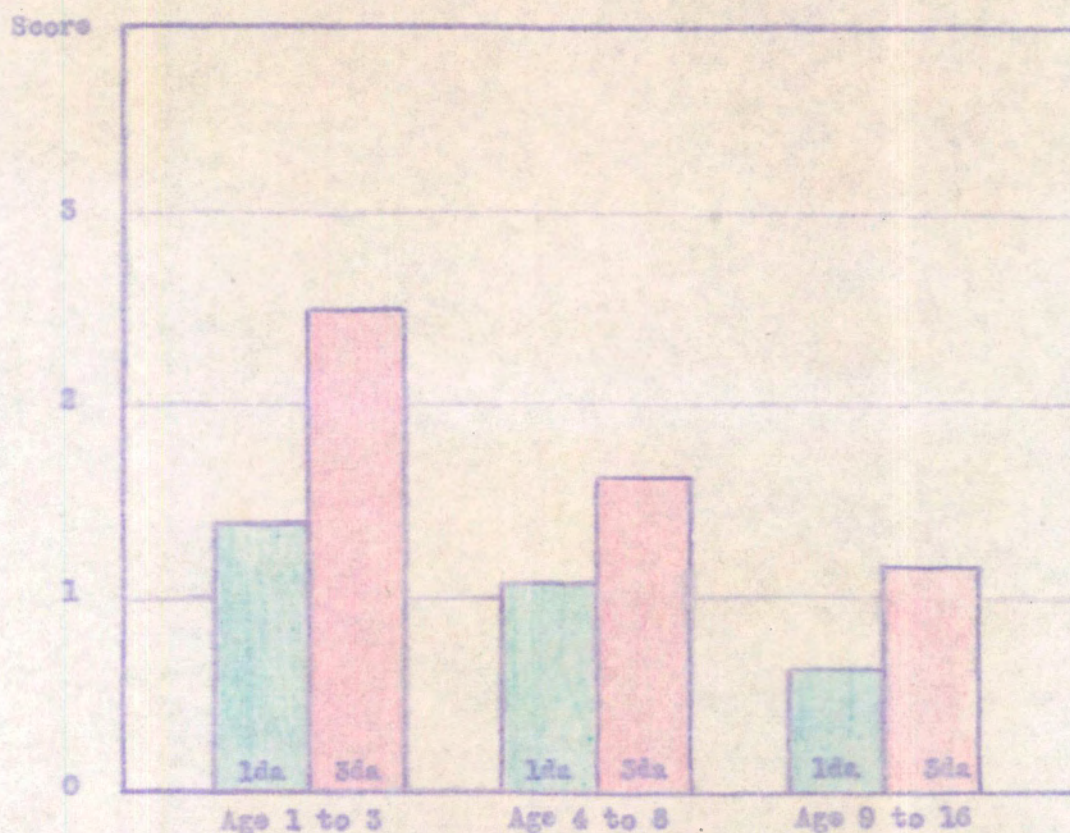


Figure 9. Average oxidized flavor score of milk from groups of cows classified as to age. January to June 1940

dation when she was on test, but as soon as she was fed less and produced less milk, her milk no longer developed an oxidized flavor. This is clearly illustrated in Figure 4 (see cow 299) when she was taken off test the latter part of May. Likewise, cow 243 produced milk that was more susceptible to becoming oxidized after she was taken from the corn silage group and placed on official test the latter part of March. It is also of interest to note Table III showing the data of the other herd studied. In this the cows on test gave milk that was very susceptible while the milk of the other cows had little tendency to become oxidized.

Age is also another factor that is thought to have some effect. For that reason Table VI was computed to give the average scores of the cows in the different age groups. This table indicates that the younger

TABLE VI. AVERAGE FLAVOR SCORE OF MILK FROM COWS CLASSIFIED AS TO AGE, JANUARY TO JUNE, 1940

Age group	First day		Third day	
	Number samples	Average score	Number samples	Average score
1 to 3 yrs.	129	1.39	133	2.50
4 to 8 yrs.	261	1.07	240	1.62
9 to 16 yrs.	94	.62	85	1.16

cows are more likely to give milk that will become oxidized. However, it must be kept in mind in considering these figures, that the younger cows are the ones usually placed on official test. Only four of the thirteen test cows are in the middle age group and only one is in the old cow group. However, it is interesting to note that Beck, Whitnah, and Martin⁽²⁴⁾

made similar findings in the examination of the milk from another college herd.

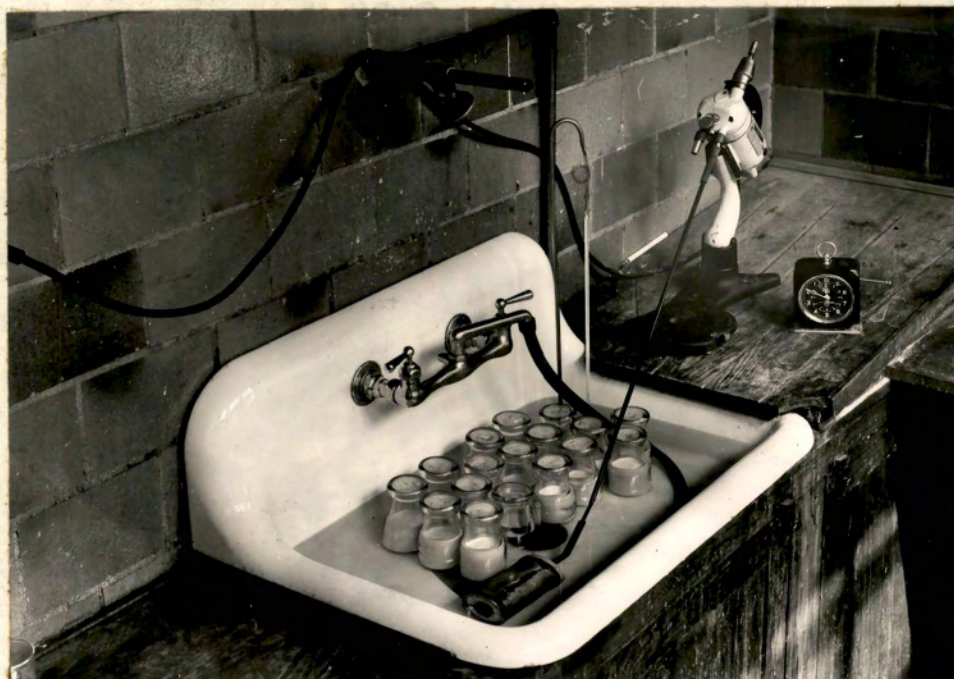


Figure 10. Pasteurizing half-pint samples of milk. Bottles are heated by hot water flowing from the tap through the rubber tube into the sink. The overflow in the center keeps the water level above the milk in the bottles. A centrifugal pump turned by an electric motor keeps the heating water circulating.

Antioxidants. Of the eleven samples of milk used to determine the effects of certain antioxidants, all produced a definite oxidized flavor in one day with the addition of copper at the rate of 2.5 p.p.m. By the fifth day all but one of the samples had developed the flavor so strong that they were unfit for use. Thus, it can be seen that the samples were susceptible to becoming oxidized and that the value of the antioxidants could be determined much easier.

Columns 3, 4, and 5 of Tables VII, VIII, and IX give the results



Figure 11. Pasteurizing two-quart samples. The hot water from the tap is forced through the rubber tube into a copper tube which circles a glass beaker filled with milk. Holes in the copper tube allow the hot water to spray on the sides of the beaker and heat the milk. The milk is stirred with a glass rod connected to an electric motor to maintain a uniform temperature throughout.

of the oxidized flavor scores of milk that was treated with varying amounts of vitamin C. In all cases it can be seen that the occurrence of oxidized flavor in these samples is not as great on any day at any concentration as is the milk that has no antioxidant added. Increasing the amounts of the vitamin in the milk seemed to lessen the flavor, especially on the fifth day, but the difference is not great enough to be of much significance since there are so few cases.

TABLE VII. OXIDIZED FLAVOR SCORE ON FIRST DAY OF MILK TO WHICH ANTIOXIDANTS HAVE BEEN ADDED

Trial	Noth- ing added	Copper added, 2.5 p.p.m.							
		No an- tiox- idant added	Vitamin C added			Sodium thiosulfate added			
			6 mgm per 100 ml	12 mgm per 100 ml	18 mgm per 100 ml	12½ mgm per 100 ml	25 mgm per 100 ml	50 mgm per 100 ml	100 mgm per 100 ml
1	0	2	1	0	0	4	2	1	--
2	0	4	2	0	0	4	4	2	--
3	0	2	0	0	0	2	1	0	--
4	0	2	0	1	0	--	1	0	0
5	0	3	1	3	1	1	1	0	--
6	0	3	1	1	3	--	1	0	0
7	0	4	2	1	2	--	1	1	0
8	0	2	2	1	1	--	0	0	0
9	0	3	1	1	2	--	0	0	0
10	0	4	2	0	0	--	2	0	0
11	0	4	1	0	1	--	1	0	0
Av.	.0	3.0	1.2	.7	.9	2.8	1.2	.4	.0

TABLE VIII. OXIDIZED FLAVOR SCORE ON THIRD DAY OF MILK TO WHICH ANTIOXIDANTS HAVE BEEN ADDED

Trial	Nothing added	Copper added, 2.5 p.p.m.							
		No antioxidant added	Vitamin C and CuSO ₄ added			Sodium thiosulfate and CuSO ₄ added			
			6 mgm per 100 ml	12 mgm per 100 ml	18 mgm per 100 ml	12½ mgm per 100 ml	25 mgm per 100 ml	50 mgm per 100 ml	100 mgm per 100 ml
1	0	3	2	1	0	3	2	1	--
2	0	4	2	3	1	4	4	2	--
3	0	4	3	3	2	4	3	2	--
4	0	1	2	3	2	--	1	0	0
5	0	4	--	4	4	3	2	2	--
6	0	4	1	3	3	--	2	2	0
7	0	3	3	2	3	--	2	1	0
8	0	2	1	1	0	--	1	0	0
9	0	4	4	4	2	--	4	1	0
10	0	4	4	3	4	--	3	2	1
11	0	4	4	4	1	--	4	2	0
Av.	.0	3.4	2.6	2.8	2.0	3.5	2.5	1.4	.1

As shown in Table IX, there is no appreciable difference in the oxidized flavor score on the fifth day due to the addition of vitamin C. Any milk that has a score of more than three would be considered objectionable by the consumer.

TABLE IX. OXIDIZED FLAVOR SCORE ON FIFTH DAY OF MILK TO WHICH ANTIOXIDANTS HAVE BEEN ADDED

Trial	Nothing added	Copper added, 2.5 p.p.m.							
		No antioxidant added	Vitamin C added			Sodium thiosulfate added			
			6 mgm per 100 ml	12 mgm per 100 ml	18 mgm per 100 ml	12½ mgm per 100 ml	25 mgm per 100 ml	50 mgm per 100 ml	100 mgm per 100 ml
1	0	3	2	2	2	3	3	2	--
2	1	4	3	4	2	4	4	4	--
3	0	4	4	3	3	3	3	2	--
4	0	4	3	4	3	--	4	2	0
5	0	4	4	4	4	3	3	3	--
6	0	4	4	4	3	--	4	3	1
7	0	4	4	4	3	--	3	0	0
8	0	4	4	3	4	--	4	2	2
9	0	--	--	--	--	--	4	--	0
10	0	4	4	3	3	--	4	3	1
11	1	4	4	4	4	--	4	3	0
Av.	.2	3.9	3.6	3.5	3.1	3.3	3.6	2.4	.6

The addition of vitamin C tablets to the milk also was responsible for an off flavor described as being "stale". Since tablets containing the vitamin C and not the pure vitamin were added to the milk, it cannot be said that the flavor was caused by the vitamin. More work must be done with the two forms to determine whether the ascorbic acid or some other part of the tablet gives this off flavor.

Because sodium thiosulfate is used in titrations of indol-phenol

to determine its vitamin C titre, it seemed that the reducing qualities of vitamin C and the thiosulfate would be about the same. Of the eleven trials made, four samples had $12\frac{1}{2}$ mgm. of thiosulfate added per 100 ml. of milk, eleven samples each had 25 and 50 mgm. of thiosulfate, and seven samples had 100 mgm. of thiosulfate added per 100 ml. of milk. The last four columns in Tables VII, VIII, and IX give the results of the addition of this antioxidant. At the end of the first four trials it became evident that the concentration of $12\frac{1}{2}$ mgm. of thiosulfate per 100 ml. of milk would not be enough to prevent the flavor development. Therefore, the amounts of sodium thiosulfate added to the rest of the samples were increased. For the first and third days, the concentration of 25 mgm. seemed to give sufficient protection, but on the fifth day oxidized flavor developed to make the milk unfit for use. In the case of 50 mgm. per 100 ml., the oxidized flavor occurring in the milk was much less even on the fifth day, but it still was noticeable in most of the cases. However, it should be noted that on the fifth day there were some of the samples that had no oxidized flavor at all, while others were very definite. The addition of 100 mgm. per 100 ml. (or .1%) prevented the flavor from becoming objectionable in all cases, even on the fifth day after the copper had been added. In all the milk that had thiosulfate added, a cooked or custard flavor was noted. This flavor seemed to disappear with the development of the oxidized flavor. Josepheson and Doan⁽⁴⁸⁾ also reported that the cooked flavor in the milk disappeared with the occurrence of oxidized flavor, which might indicate that some form of a thiosulfate is formed to give milk its cooked flavor.

SUMMARY

Thirty-eight per cent of the samples of milk taken, in this investigation, from eleven milk distributors in Knoxville, Tennessee, during the winter of 1939-40, were found to have an oxidized flavor. The samples from four plants were frequently oxidized while the others had few cases of oxidized milk.

A survey of milk from different herds furnishing the University Creamery with milk, indicated that there is a great variance in the copper-induced oxidized flavor from different herds. This variance was also noted in the milk from different cows of the University herd.

When the cows of the University herd were classified as to the method of feeding, those cows on official butterfat test ration produced milk much more susceptible to oxidation than other cows in the same herd. Little difference was apparent in the groups of cows fed corn, alfalfa, or sericea silages. When classified as to age, the younger cows produced milk that was more susceptible to oxidation than the others. Whether this is due to the difference in age or that the heifers were, in most cases, placed on official test, is not known.

The addition of vitamin C tablets at the rate of 6 mgm., 12 mgm., and 18 mgm. per 100 ml. helped to prevent the development of oxidized flavor up to the fifth day, at which time little effect was noted.

The addition of sodium thiosulfate at the rate of $12\frac{1}{2}$ mgm., 25 mgm., 50 mgm., and 100 mgm. per 100 ml. prevented the flavor on the first day, but only the 100 mgm. per 100 ml. prevented the flavor on the fifth day. The sodium thiosulfate gave the milk a cooked or custard taste

which disappeared when the oxidized flavor developed.

During the work on this project several questions have come up that deserve further consideration. Some of these are of such importance that they could be used for developing a further research problem.

1. Is beet pulp the cause of oxidized flavor? Would the substitution of citrus pulp eliminate the difficulty with test cows?
2. Does the high production level of cows have any effect on the occurrence of oxidized flavor in milk?
3. What are the best antioxidants to use?
4. What correlation is there between color and carotene in milk and the occurrence of oxidized flavor?
5. How does the feeding of certain feeds rich in vitamins A and C affect the occurrence of oxidized flavor?
6. Would it be possible to eliminate trouble with oxidized milk by use of higher pasteurization temperatures?

Since the problem of oxidized flavor in milk is so great and this investigation was started only one year ago, it does not seem wise to attempt to draw any general conclusions at this time. However, as a result of this work, there were several indications as to the cause and prevention of oxidized flavor. The above questions attempt to point the way for a further study of these with the hope that work will be continued along some of these specific lines.

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