# THE EFFECT OF THE COMPOSITION OF BUTTERFAT ON ITS SUSCEPTIBILITY OF OXIDATION*

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OXIDATIVE rancidity, as found in milk and dairy products, has been characterized by a number of terms such as "emery", "metallic", "astringent", "papery", "cardboard", "mealy", "oily" and "tallowy". The difference in flavor probably represents various stages in the oxidation of the fat as well as the conditions under which the oxidation occurred. Light and metals, especially copper and iron, catalyze the oxidation of butterfat, yet tallowy or oxidized flavors are often found in milk from herds where utmost precautions have been taken to produce clean milk and to protect it from light and from metal contamination. In such high quality milk where the bacterial content is low and conditions are unfavorable for bacterial growth the oxidation-reduction potential of the milk remains relatively high and the oxidation of the fat is favored.

Trouble with this off-flavor frequently occurs spasmodically, appearing for a time and then disappearing for no apparent reason. In such cases the trouble is usually traceable to the milk from one or more cows of the herd. Guthrie and Brueckner (1) and Chilson (2) reported that 21 to 30 per cent of the cows from the herds examined gave milk during the winter and spring months which developed an oxidized flavor. Of the 349 milk samples entered at the California State Fairs from 1930 to 1934, 24.2 per cent were criticized as having an oxidized flavor.

This difference in the susceptibility of the butterfat to oxidation from individual cows is not only found in the milks but also in the butter made from these milks. The composition of butterfat is by no means fixed, but has been found to vary with the individual cow and with the food which she consumes. These observations would suggest that the difference in the susceptibility to oxidation of various butterfat samples is probably to be found in the composition of the fat.

### Experimental Procedure

It is now quite universally accepted that the formation of peroxides is the first stage in the oxidation of a fat and that the peroxide oxygen increases as the oxidation proceeds. The amount of peroxide oxygen present in a fat serves as an excellent criterion of the degree of oxidation of that fat.

Fresh butterfat contains no peroxides but when conditions favorable for oxidation are maintained peroxides begin to form, slowly at first, until a certain point is reached, after which the peroxide formation is very rapid. This period of slow peroxide formation or induction period varies in length a great deal with different butterfat samples and is a measure of the stability of the fat toward oxidation.

Figure 1, showing the formation of peroxides, the bleaching of the butterfat, and the development of volatile acids, is typical of the oxidation of fresh butterfat. With this particular sample of fat the presence of peroxides was first detected at the end of 3 hours, at which time the peroxide number of the fat was 0.05. The peroxides continued to develop slowly up to 25 hours, the end of the induction period, and then rapidly until a maximum peroxide number of 166.1 was reached at the end of the 160th hour. After this period the melted fat was becoming noticeably more viscous than it was in the beginning.

The bleaching of the color was first observed at the end of 4 hours and was then quite rapid for the next three hours, after which it did not change until after the 26th hour when there was another period of rapid bleaching. The color then remained constant for 20 hours after which there was a gradual bleaching to the 106th hour. At this time it was equivalent in color to a solution containing 0.1 millimoles of potassium dichromate per liter. This melted fat retained the faint tinge of yellow until the end of the oxi-

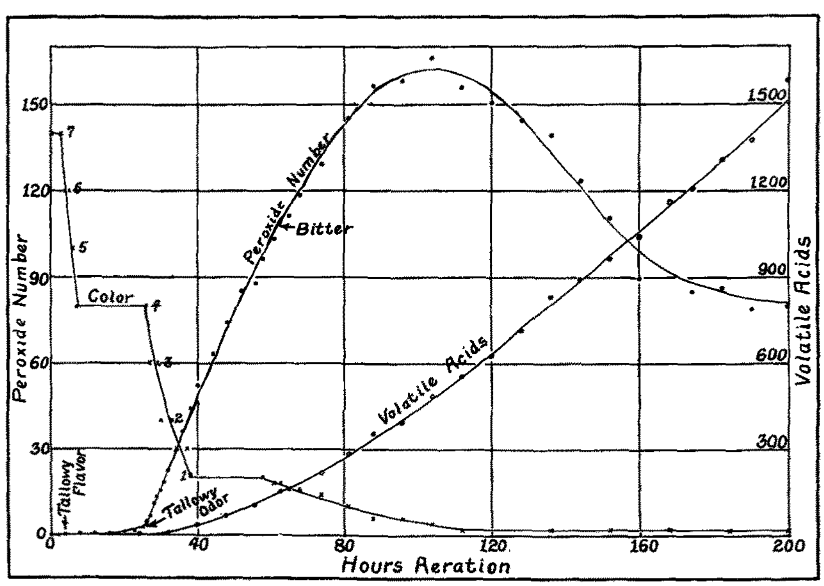


**Figure 1. The Oxidation of Butterfat by Aeration and Heat.**

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dation period, although the solidified fat was white. The color of the melted fat is expressed in terms of millimoles of potassium dichromate per liter of water solution.

The development of volatile acids was slow until after the 30th hour when the increase became quite rapid to the end of the oxidation period. As might be expected, the rapid increase in volatile acids occurred in a few hours after the rapid rise in peroxide as the acids are the result of a secondary reaction after the formation of peroxides. The amount of volatile acids are expressed as the number of cubic centimeters of N/14 acid produced from one kilogram of butterfat.

The end of the induction period of butterfat is well marked by the rapid bleaching of the fat, the rapid increase in peroxide value and the subsequent increase in volatile acids. This latter fact has been used by the authors (3) for determining approximately the end of the induction period with the accelerated Swift Fat stability test.

At the end of the 2nd hour the fat had a slight off-flavor, but was not tallowy. A slight tallowy flavor appeared at the end of the third hour at which time the first positive test for peroxides was obtained. However, in other trials, the tallowy flavor did not always appear at the same time the first test for peroxide was obtained, but varied with the different samples of butterfat. In some instances a peroxide number of 6 or higher was reached before the fat was distinctly tallowy. A tallowy odor was not evident until the end of the induction period.

In these trials the susceptibility of the butterfat to oxidation, as measured by the length of the induction period, was determined with an accelerated fat stability apparatus (3) similar to the one developed in the Swift Laboratories (4) for measuring the stability of lard.

In making stability tests, three 20 cc. samples of the same butter oil were placed in the oxidation apparatus in succession at hourly intervals and the approximate end of the induction period determined by absorbing the volatile acids carried over by the aeration in an alkaline solution containing 1 cc. of N/100 sodium hydroxide. The indicator used was methyl red and when the change was from yellow to red in the tube which was started first, that sample of fat was beyond the end of the induction period and had a peroxide number greater than 10. The other two samples which had been in the apparatus for one and two hours less were still in the induction period or nearing the end. All three samples were removed at this point and analyzed for peroxides. It was found necessary to use great care in cleaning the glassware which came in contact with the fat in making stability tests. After thoroughly washing the tubes and soaking them in chromic acid cleaning solution over night, the best results were obtained by leaching the tubes in several changes of distilled water over a period of three or four days.

As used in this paper, the stability value of butterfat may be defined as the number of hours required to oxidize 200 cc. of butter oil at 100° C. to a peroxide value of 10 when air is bubbled through the fat at the rate of 2.33 cc. per second. The peroxide value of 10 was selected more or less as an arbitrary value as marking the end of the induction period as this value lies near the beginning of the period of rapid peroxide formation.

The color of the fat was determined by comparison with standard solutions of potassium dichromate and is expressed as the number of millimoles of potassium dichromate per liter of solution.

The refractive index of the fat was measured with a Zeiss butyrorefractometer at 40° C. The iodine number was determined according to the Hanus method.

The thiocyanogen-iodine number was determined according to the directions given by Jamieson (5) except that 0.5 gram of fat and 50 cc. of the thiocyanogen solution was used. It was found that with butterfat these larger amounts of fat and thiocyanogen solution gave better results. These findings are in accord with those of Wiley and Gill (5) who recommend 0.4 gram samples and 50 cc. of the thiocyanogen solution.

The percentages of free fatty acids were calculated according to the following formulae:

(X) Per cent linoleic acid $= 1.104 \times (\text{I. No.} - \text{SCN No.})$

(Y) Per cent oleic acid $= 1.112 \times (2\ \text{SCN} - \text{I. No.})$

Per cent saturated acids + unsaponifiable matter $= 95.7 - (X + Y)$

In view of the findings of Bosworth and Brown (6) in which they report that their attempts to verify the occurrence of ordinary linoleic acid failed but that they found other acids with two double bonds and also highly unsaturated acids of the arachidonic type, these calculations are probably not exact but serve to indicate in a comparative way the amounts of acids with unsaturation greater than oleic acid.

## Experimental Results

### A. The Stability of Creamery Butter

In order to obtain some idea as to what could be expected as to the stability value of butter fat, a number of butter samples sent to the University Dairy Department from various creameries for scoring as well as the butter from the University Creamery were tested. The stability values as well as the scores of the butter given in Table 1. As might be expected there was no direct correlation between the score and the stability value, however, it is of interest to note that the lowest scoring sample had the lowest stability value. From these results it appears that fresh creamery butter has a stability value of approximately 20.

TABLE 1.—STABILITY VALUE OF VARIOUS SAMPLES OF CREAMERY BUTTER

| Sample | Score | Stability Value |
|---|---|---|
| University Creamery | | 20½ |
| University Creamery | | 19 |
| 216 | 86 | 8 |
| 213 | 90 | 21 |
| 209 | 89½ | 18 |
| 207 | 92½ | 20½ |
| 210 | 91½ | 18 |
| 212 | 90½ | 20 |
| 214 | 90½ | 19 |
| 211 | 92 | 14 |
| 208 | 92 | 14 |

### B. Effect of Breed and Individuality of the Cow

Samples of milk were obtained from a number of cows of the University herd while the cows were all receiving the same winter ration. The ration was quite characteristic of the average Wisconsin dairy herd ration and is given at the bottom of Table 2.

There appears to be a great deal of variation between the individual cows of the same breed and from breed to breed. With the small number of cows tested averages have but little value, but it seems quite definite that in general the milk fat from the Holstein cows on winter rations was more susceptible to oxidation than that from the other breeds. The milk fat from most of the Guernsey cows had an exceptionally high stability value, one as high as 54½.

No opportunity was afforded at the time to check the stability of the fat from day to day as the ration of most of the cows was changed shortly after the first samples were obtained. However, two cows, M87 and Lotus, were kept on winter rations and their fat was again tested after about an interval of one month. The fat of M87 showed stability values of 29½ and 15 respectively and that of Lotus 36 and 40½. These values would seem to indicate that the stability of the fat is subject to variations even though the cow is kept on the same ration.

**C. Effect of Feed**

When the cows were placed on a summer ration where grass replaced the alfalfa hay and the oil meal was omitted from the grain ration, the stability values dropped in all cases except two (see Table 2, cows 67 and Fuschia) and in these two cases the values obtained for the fat on the winter ration seem to be low as compared to those of the other cows. When the averages of the cows in the various breeds were compared on summer ration the values were so close that it may be concluded that there was no difference.

TABLE 2.—EFFECT OF BREED AND INDIVIDUALITY OF COW

| Breed | COW | WINTER RATION Stability Value | SUMMER RATION Stability Value |
|---|---|---|---|
| HOLSTEIN | | | |
| | 65 | 42 | 24 |
| | 67 | 19½ | 26½ |
| | 68 | 30½ | 24½ |
| | M87 | 29½ | .... |
| | Average for Breed | 30.4 | 25 |
| GUERNSEY | | | |
| | 404 | 51 | 38 |
| | 432 | 42½ | 17½ |
| | 433 | 31 | 24½ |
| | 435 | 41½ | 25½ |
| | 436 | 54½ | 22 |
| | Fawn | 42 | 26 |
| | Average for Breed | 43.7 | 25.7 |
| AYRSHIRE | | | |
| | Janice | 27½ | 29½ |
| | Quintilla | 52 | 30 |
| | Lass | 18 | 17¼ |
| | Average for Breed | 39.2 | 25.6 |
| BROWN SWISS | | | |
| | Fuschia | 22½ | 27 |
| | Fidelity | 48 | 27 |
| | Dora | 46½ | 20½ |
| | Average for Breed | 39.0 | 24.8 |
| JERSEY | | | |
| | Lotus | 36 | .... |

HERD RATIONS

| Winter Ration: | Summer Ration: |
|---|---|
| Alfalfa Hay | Grass |
| Corn Silage | Corn Silage—1 feed |
| Grain: | Grain: |
| 500 corn and cob | 700 corn and cob |
| 200 oats | 200 bran |
| 200 bran | 100 oats |
| 100 oil meal | |

**D. The Relation of Various Fat Constants to Butterfat Stability**

For these trials, eight cows were selected, two from each breed—Guernsey, Jersey, Holstein and Brown Swiss. These cows were all in the early stages of their lactation period but differed in their ages. The data on the stability values and fat constants of the butterfat are given in Table 3.

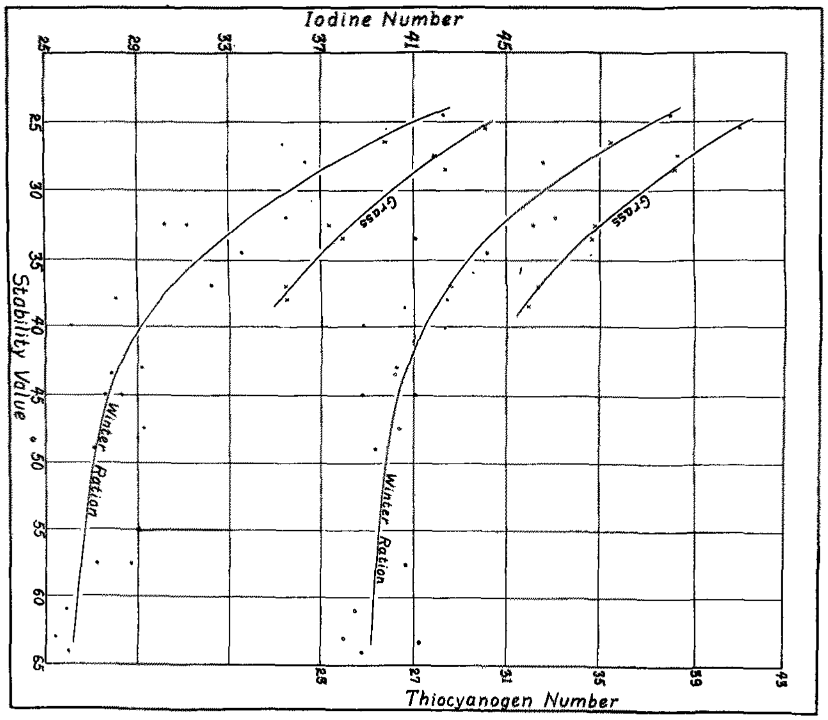


**Figure 2. The Relationship Between the Unsaturation and Stability of Butterfat.**

Whether carotene acts as an atioxidant or prooxidant is still a debated question. The view that carotene acts as a prooxidant is taken by Olcovich and Mattill (7) and Greenbank (8), while Monogahn and Schmitt (9) found that carotene greatly inhibited the oxygen uptake of linoleic acid but after the carotene had been oxidized it slightly accelerated the oxygen uptake of this acid. From the results reported in Table 3 it may be seen that there is no correlation between the carotene content as measured by the color of the butterfat and its stability toward oxidation.

The iodine and thiocyanogen numbers show that there is a distinct relationship between the unsaturation of the fat and its stability. In general, the greater the un-

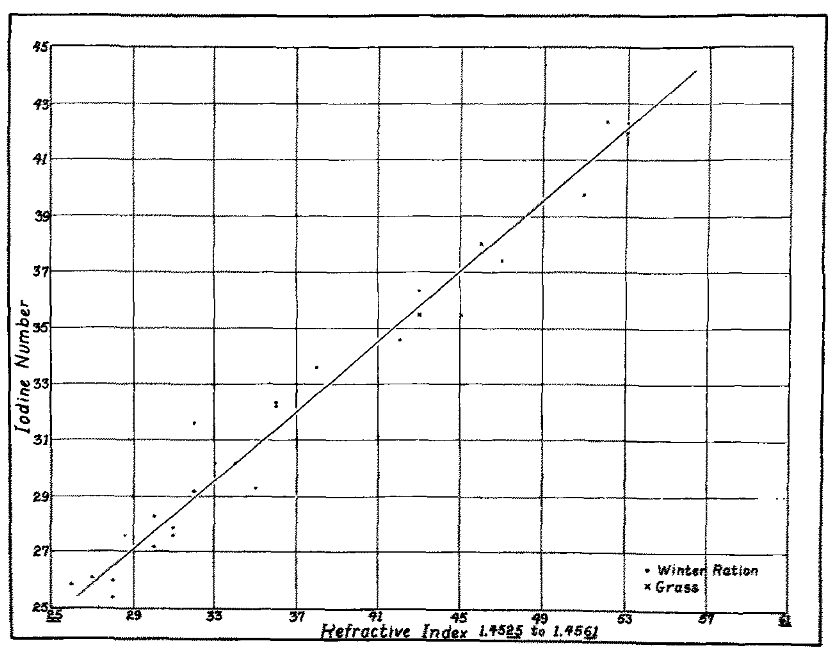


**Figure 3. The Relationship Between the Iodine Number and Refractive Index of Butterfat.**

TABLE 3.—RELATION OF VARIOUS FAT CONSTANTS TO STABILITY

| Cow | Ration | Stability Value | Color of Fat | Refractive Index | Iodine No. | Thio-cyanogen No. | Linoleic Acid (%) | Oleic Acid (%) | Sat. Acids (%) |
|---|---|---|---|---|---|---|---|---|---|
| (February 29, 1936) | | | | | | | | | |
| Chum | I | 64 | 6 | 1.4528 | 26.0 | 24.8 | 1.33 | 26.3 | 68.1 |
| Dora | I | 61 | 6 | 1.4526 | 25.9 | 24.5 | 1.55 | 25.7 | 68.5 |
| Fidelity | I | 57½ | 2 | 1.4533 | 28.7 | 26.7 | 2.21 | 27.5 | 66.0 |
| Lotus | I | 57½ | 5 | 1.4530 | 27.2 | 26.7 | 0.56 | 29.2 | 65.9 |
| Fuchsia | I | 45 | 3 | 1.4531 | 27.6 | 24.8 | 3.10 | 24.5 | 68.11 |
| Bess | I | 38 | 2 | 1.4533 | 28.1 | 28.4 | .... | .... | .... |
| Safety Belle | I | 32½ | 5 | 1.4536 | 32.2 | 30.2 | 2.21 | 31.4 | 62.1 |
| Rosette | I | 32 | 3 | 1.4543 | 35.5 | 33.1 | 2.66 | 34.2 | 58.8 |
| (April 7, 1936) | | | | | | | | | |
| Lotus | II | 63 | 5 | 1.4528 | 25.4 | 24.0 | 1.55 | 25.2 | 69.0 |
| Dora | II | 49 | 6 | 1.4526 | 27.1 | 25.4 | 1.88 | 26.4 | 67.4 |
| Safety Belle | II | 45 | 4 | 1.4530 | 28.3 | 27.1 | 1.33 | 28.8 | 65.6 |
| Rosette | II | .. | 2 | 1.4542 | 34.6 | 32.2 | 2.65 | 33.2 | 59.9 |
| Fidelity | II | 43½ | 2 | 1.4531 | 27.9 | 26.2 | 1.88 | 27.3 | 66.5 |
| Chum | II | 40 | 6 | 1.4527 | 26.1 | 24.8 | 1.44 | 26.2 | 68.1 |
| Fuchsia | II | .. | 2 | 1.4529 | 29.0 | 27.6 | 1.55 | 29.2 | 65.0 |
| Bess | II | .. | 2 | 1.4533 | 30.2 | 27.2 | 3.32 | 27.0 | 65.4 |
| (May 5, 1936) | | | | | | | | | |
| Chum | III | 47½ | 5 | 1.4535 | 29.3 | 26.4 | 3.21 | 26.2 | 66.3 |
| Safety Belle | III | 43 | 3 | 1.4532 | 29.2 | 26.3 | 3.21 | 26.1 | 66.4 |
| Fuchsia | IV | 37 | 1½ | 1.4536 | 32.3 | 28.6 | 4.09 | 27.7 | 63.9 |
| Fidelity | IV | 34½ | 1½ | 1.4538 | 33.6 | 30.2 | 3.75 | 29.8 | 62.2 |
| Lotus | III | 33½ | 6 | 1.4534 | 30.2 | 27.1 | 3.42 | 26.8 | 65.5 |
| Bess | IV | 28 | 1 | 1.4543 | 36.3 | 32.6 | 4.09 | 32.2 | 59.4 |
| Rosette | IV | 24½ | 1½ | 1.4553 | 42.3 | 38.0 | 4.76 | 37.6 | 53.3 |
| Dora | III | .. | 6. | 1.4532 | 31.6 | 27.8 | 4.20 | 26.7 | 64.8 |
| (May 19, 1936) | | | | | | | | | |
| Lotus | V | 38 | 7 | 1.4545 | 35.5 | 32.0 | 3.87 | 31.7 | 60.1 |
| Dora | V | 37 | 8 | 1.4543 | 35.5 | 32.4 | 3.43 | 32.6 | 59.7 |
| Safety Belle | V | 33½ | 7 | 1.4546 | 38.0 | 34.7 | 3.65 | 35.0 | 57.1 |
| Chum | V | 32½ | 10 | 1.4547 | 37.4 | 34.8 | 2.88 | 35.9 | 56.9 |
| Fuchsia | V | 28½ | 2½ | 1.4552 | 42.4 | 38.2 | 4.65 | 37.9 | 53.2 |
| Fidelity | V | 27½ | 2½ | 1.4553 | 41.9 | 38.3 | 3.98 | 38.6 | 53.1 |
| Bess | V | 26½ | 2 | 1.4551 | 39.8 | 35.4 | 4.87 | 34.5 | 56.3 |
| Rosette | V | 25½ | 2½ | 1.4561 | 44.1 | 41.0 | 3.43 | 42.2 | 50.1 |

Rations—Mixed hay throughout

| I. | II. | III. | | IV. | V. |
|---|---|---|---|---|---|
| Corn Silage | Corn Silage | Corn-alfalfa | A.I.V. Silage | Same as III except molasses silage instead of A.I.V. | Cows on grass May 11 |
| 5—corn | corn-alfalfa silage | One Feed | One Feed | | Alfalfa mixed hay |
| 2—oats | 5—corn | 5—corn | 5—corn | | Small amount of A.I.V. alfalfa silage |
| 2—bran | 3—oats | 3—oats | 5—oats | | 5—corn |
| 1—oil meal | 2—bran | 2—bran | | | 5—oats |
| | | | | | 5—bran |

saturation the less stable is the fat. When the values for the iodine number and thiocyanogen number are plotted against stabilities as shown in Figure 2, two distinct curves result; one for the values obtained when the cows were on winter rations and one when they were receiving green grass. These results show that even though the fat was more unsaturated and less stable when the cows were receiving grass, the fat was more stable for the same degree of unsaturation than when the cows were receiving winter rations. The changes brought about by grass were more pronounced in the butterfat from cows where the iodine number had been low and the stability high when on dry feed.

As shown in Figure 3, a very close relationship was found to exist between the iodine number of butterfat and its refractive index. As the iodine number of the fat increased the refractive index increased. If refractive indices are plotted against stability values, curves are obtained which are similar to those in Figure 2.

Figure 4 shows the curves ob-

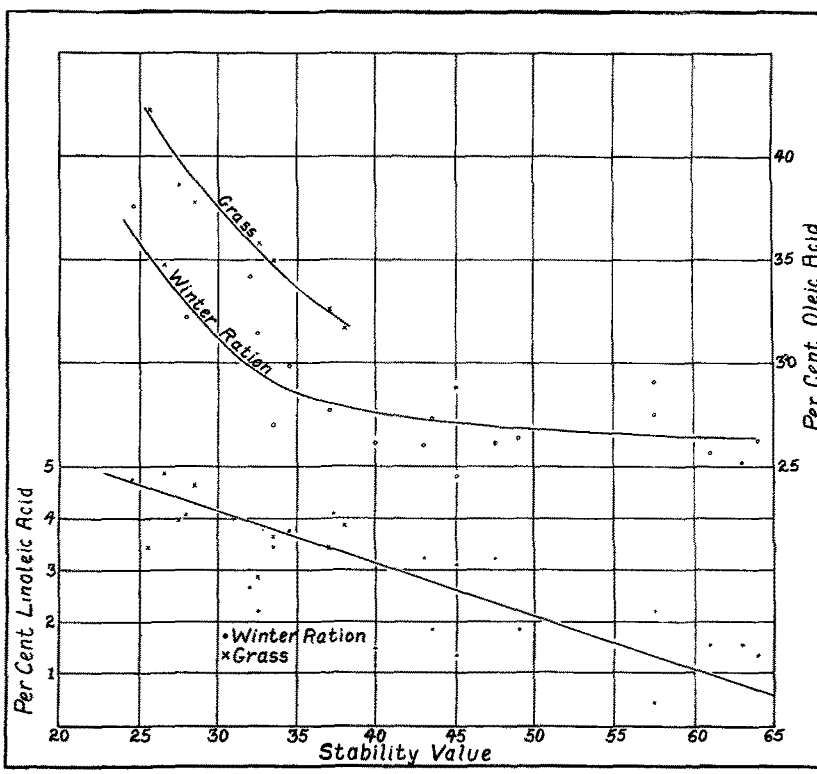


**Figure 4. The Relationship Between the Amount of Oleic and Linoleic Acids to the Stability Value of Butetrfat.**

tained by plotting the percentages of oleic and linoleic acid against stability of the various samples of butterfat. In the case of oleic acid, two distinct curves are produced similar to those in Figure 2, one for the fat when the cows were on dry feed and the other when the cows received grass. The percentage of linoleic acid appears to be a straight line function of the stability of the fat; decreasing as the stability of the fat increases. The fact that the linoleic acid values for the butterfat from the cows on grass fall on the same curve as the value for the butterfat from the cows on winter rations leads to the conclusion that the percentage of linoleic acid determines the stability.

The scattering of the points on these curves is due, no doubt, to the fact that it is impossible to measure the stability of the fat with the same degree of accuracy as the iodine and thiocyanogen number.

## DISCUSSION

The results of these trials indicate that the stability of the fat is influenced by its unsaturation and are in accord with those of Henderson and Roadhouse (10) who found that the fat from cows on submaintenance rations was more unsaturated and more susceptible to oxidation.

In trials covering the spring periods of 1935 and 1936 the authors have shown that when the cows were turned out on grass the fat became more susceptible to oxidation. These results seem to be contrary to practical experience and scientific observations (1), (11) that the milk from cows on grass is less apt to develop oxidized flavors than when on dry feed. These findings lead the authors to believe that there are protective substances in milk which become more plentiful when the cows are on grass and which prevent the oxidation of the fat even though the separated butter oil is more susceptible to oxidation. The presence of protective substances might explain why Frazier (12) was unable to determine any difference in the development of a tallowy flavor in the milk of cows fed a ration which should have caused the fat to be less saturated and more subject to oxidation.

From the data obtained in these trials it is impossible to attribute the variations in the stability of the milk fat of the various cows or of the same cow at different periods to the age of the cow or the stage in her lactation period.

## SUMMARY

There is considerable variation in the stability of the butter fat toward oxidation where it is obtained from different cows and from an individual cow at different periods.

The stability of butterfat toward oxidation bears an inverse relation to the unsaturation of the fat.

The fat from cows receiving grass as part of their ration is less saturated and more susceptible to oxidation.

It appears that the amount of linoleic acid rather than the oleic acid governs the stability of butterfat.

The results point to the presence of protective substances in milk in increased amounts when cows are on grass which prevent the development of oxidized flavors in the milk. There is no relation between the carotene content, as evidenced by the color of the fat, and the stability of the fat toward oxidation.

The refractive index of butterfat varies in direct proportion to the iodine number of the fat.

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# A Brief Study of Indicators In Determining Free Fatty Acids In Dark Colored Oils

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THIS study embraces four indicators, phenolphthalein, which is used according to the present official method; methyl blue, which was official many years ago; thymol blue, which is chemically thymolsulphonephthalein, and thymol phthalein. The last two were suggested by Dr. Herbert A. Lubs, one of the country's greatest authorities on indicators, after presenting the problem to him. There is no question about our having a problem because whenever there is a wet season, dark oils are produced and since these dark oils also have a reddish cast, titration to the end-point shown by phenolphthalein is often very obscure and indefinite, especially when the free fatty acids appreciably exceed 12 per cent, but the point at which the more or less indefinite realm begins varies with individuals. Some plant chemists