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 endpoint 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 have held to methyl blue, while others have used methyl blue in making a pilot titration to be followed with phenolphthalein.

The purpose of my discussion is to show that we may select an indicator that is better adapted for the determination in dark oils, and which is equally as good as phenolphthalein in the case of light colored oils, and which meets all of the necessary requirements such as uniformity, stability and sharpness of end-point, etc., and owing to the marked contrast in the color change, the end-point may be rendered far more definite, and consequently the results far more reliable in the case of the very dark oils. I refer particularly to thymol blue. When this indicator is added to the alcohol and neutralized, the color change from the bright canary yellow to blue is equally as sharp or sharper than phenolphthalein, and it shows up better and owing to the marked contrast, the color reaction at the final stage is quickly and easily perceived even when working on very dark oils. To illustrate this, I made an experiment by taking approximately four times the prescribed amount of oil, or about twenty-eight grams whose free fatty acid content was 11 per cent, and I obtained a fairly good end-point. This was about equivalent to taking 7.05 grams of oil that contained 44% free fatty acids, and therefore, this was a largely exaggerated experiment.

The alkaline pH range of thymol blue is fairly close to that of phenolphthalein, being from 8.0 to 9.6 as against 8.5 to 10.5 and results will be found to check. For instance, I found a sample to show 12.6% when using both indicators. The strength of the thymol blue was 0.04% in alcohol, and from two to five c.c. were added to the fifty c.c. of alcohol and neutralized to the blue color in the same manner in which phenolphthalein is used.

Thymol Phthalein

After making various determinations and experiments, I found this to be much more satisfactory than phenolphthalein when working on dark oils, but not as satisfactory as thymol blue, and the end-point is not as sharp. The pH range of this is much like phenolphthalein, being from 9.2 to 10.4, and my result found by its use on a sample was 12.7% as against 12.6% when using phenolphthalein.

Methyl Blue

Methyl blue is mentioned in some books as being sodium triphenyl-para-rosaniline sulfonate and Dr. Lubs says this probably is closely related to "Poirriers" blue.

Though this used to be one of the official indicators of the Society, its use has long since been discarded in official work. According to my experiments, however, good results may be obtained by its use, and the color change from blue to red is observed at once even when titrating dark oils. The end-point is sharp, but I believe that some have found that the uniformity of this compound cannot always be depended on. I made some experiments with the contents of three small bottles. One was probably several years old, one not so old, and one had just been purchased. All were manufactured by The Coleman & Bell Company, Norwood, Ohio. I found by experiment that the strength varied and that the oldest was the weakest. The lot just purchased came in the form of crystals about the size of those of potassium permanganate, while the other two lots were more like powder. I found, however, that in making actual determinations, the same results were obtained throughout, and this was 12.6%, the same as when phenolphthalein was employed.

There is one thing about this indicator which may have helped to cause prejudice against its use, which I shall mention, and that is the fact that after neutralizing the alcohol, it will show an acid reaction by a complete change in color in the presence of appreciably more CO_2 than that normally present in air, namely 0.04%, so that when a number of Bunsen burners are turned on, the atmosphere in the laboratory is sufficiently charged with CO_2 (especially with poor ventilation), and naturally the same atmosphere exists in the flask in which the titration is conducted, which causes a disturbance in the indicator, so that when the fifty c.c. of alcohol in the bottom of the flask is neutralized, the atmosphere in the flask above the liquid is not, but by shaking the alcohol in the same way as is done when the oil is titrated, the alcohol will collect the CO_2 from the flask atmosphere and ultimately neutralization will be accomplished. This idea fits in with the method of measuring the 7.05 grams, but not with the official method of weighing it. The effect of the atmosphere caused by a Bunsen burner may be at once seen by holding an inverted flask over the flame for a second and then adding the neutralized alcohol and titrating. I also found by experiment that the flask atmosphere affects all of the other indicators included in this study to a more or less degree.

To illustrate the effect of CO_2, an experiment was made by inflating the lungs once and blowing through a glass tube into fifty c.c. of neutralized alcohol containing the various indicators, and the amounts of N/4 alkali required to bring back to the same point were found to be as follows:

Phenolphthalein	2.0 c.c.
Thymol blue	2.0 c.c.
Methyl blue	2.5 c.c.
Thymol phthalein	3.0 c.c.

When it is considered that the capacity of one's lungs is approximately a liter, and that the exhaled air contains from 3.7 to 5.5% of CO_2 (Stitt's Laboratory Manual on biological chemistry) and that we take a liter to weigh 1.43 grams, the amount of CO_2 that would be equivalent to two per cent of free fatty acids, as far as titration is concerned, would be only about 0.06 gram on the basis of 7.05 grams of oil. Therefore, I think the CO_2 flask atmosphere should be checked whenever there is doubt, and the proper correction made. Obviously, the smaller the titrating vessel, all other things being equal, the less is the effect of CO_2. A regular four ounce oil sample bottle seems particularly well adapted for the purpose, and the end-point is more easily seen than when an Erlenmeyer flask is used.

Finally, I would like to say a word about the addition of ten c.c. of petrolic ether. The present rule book of the National Cottonseed Products Association directs this when making the determination of the free fatty acids in the oil in making a seed analysis, but it is omitted, probably unintentionally, in making the determination in crude oil. I think the petrolic ether should be, by all means, added in all cases as this greatly facilitates the titration on account of affording closer contact between the free fatty acids and the N/4 alkali, and as a result much less shaking is necessary and the end-point is sharper by reason of the better contact.