THE RELATIONSHIP OF THE LECITHIN CONTENT OF MILK TO THE DEVELOPMENT OF OXIDIZED FLAVOR

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Wm. K. Fox

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INTRODUCTION

Many "off" flavors have been encountered during the past few years in market milk. Most of these flavors have been found to have gained entrance to the milk through one or more of the following avenues: (1) feeds fed to the cow; (2) direct absorption of surrounding odors; (3) bacterial growth; (4) variations in the actual chemical composition of the milk; and (5) chemical action.

Those flavors developing through chemical change probably cause the greatest concern. The most important, and undoubtedly the least understood, of the "off" flavors in milk due to chemical action has been the "oxidized" flavor.

The oxidized flavor in milk is not new. The very fact that numerous names, eg., "papery," "cardboardy," "cappy," "oily," "tallowy," "suety," "emery," and "metallic," have been applied to this flavor at various times merely shows how widespread and confusing the oxidized flavor has been and remains up to the present. Confusion in nomenclature pertaining to this flavor has made the correlation of the work of European and American investigators on the subject difficult. However, much investigational work points to the fact that oxidized flavor is not caused by a single factor, but probably by several factors.

Although a search of the literature shows that a great amount of research has been done pertaining to the various causes of oxidized flavor, little work has been undertaken concerning the effect of certain feeds on the lecithin content of milk and, hence, its relation to the oxidized flavor. This thesis records investigational work chiefly on that phase of the problem.

REVIEW OF LITERATURE

Introduction. As early as 1905 attention was first directed to the oxidized flavor in milk by Golding and Feilmann (12). Guthrie (14) pointed out, however, that prior to this time White, in 1901, had designated a "metallic" flavor in butter. Further citations by Guthrie indicated clearly that the "oxidized" flavor was mistaken for some time for this "metallic" flavor. Recent research by Roland, Sorensen, and Whitaker (34) indicated the high prevalence of oxidized flavor in commercial bottled milk particularly in the high fat, premium quality product.

Since the oxidized flavor was first identified there have been numerous studies made on the subject by foreign as well as by American investigators. The quest has been to find the definite cause of the oxidized flavor, whether it be called papery, cardboardy, cappy, oily, or oxidized.

Research has shown that many factors, operating singly or in combination, affect the susceptibility of milk to oxidized flavor development. A review of the literature pertaining to the relationship of various factors to the development of the oxidized flavor is herein given.

Effect of ascorbic acid, vitamin C, content of milk on oxidized flavor. Chilson (8) found that the addition of ascorbic acid (cebione) had a marked retarding effect on the development of oxidized flavor.

Sharp, Trout, and Guthrie (37) did some recent work on oxidized flavor and vitamin C. They stated that no relationship was found between the variations in the original ascorbic acid content of the fresh milk and the development of the oxidized flavors, but a very definite relationship was found between the rate of disappearance of the ascorbic acid and the development of the oxidized flavor of milk. They also found that the addition of ascorbic acid had a tendency to prevent the development of oxidized flavor.

A recent report from the West Virginia Agriculture Experiment Station (10) carried the following comment. "Evidence that the presence of vitamin C prevents the development of oxidized flavor was obtained by feeding tomato juice in the amount of two quarts daily to three cows known to be producing susceptible milk. The susceptibility diminished and disappeared after several weeks of this treatment, but recurred when the feeding of tomato juice was discontinued. Similar results were also obtained from the feeding of pure vitamin C (cebione) and lemon juice. Likewise, in the case of six cows which produced susceptible milk on a dry barn feeding regime, the susceptibility disappeared when the animals were on pasture."

Riddell and others (32) found that milk from cows fed either on pasture or on dry feed showed no significant biological or chemical difference in vitamin C content. Whitnah, Riddell, and Caulfield (49) added 0.3 gram of copper daily to the ration of certain cows and found that in the case of these cows it produced a significant decrease in the stability of the vitamin C in pasteurized milk and assumed there would be a corresponding increase in copper content of the milk. Normal variations of copper in the ration, they stated, would probably not produce any significant effect on the vitamin C of milk.

Rasmussen and others (30) studied the effect of breed characteristics and stages of lactation on the vitamin C content of cows milk. They observed that after the first two months of lactation the ascorbic acid content of the milk secreted depended solely upon the ascorbic acid content of the diet of the cow. They stated that in view of a constant intake of ascorbic acid in the diet of the cow and a decrease in milk production, such as usually occurs in the later stage of lactation, one might expect milk of higher vitamin C content toward the end of the lactation period especially if the storage capacity of the cow for this substance is limited. They found that mastitic milk gave abnormal ascorbic acid values. They also presented tables which showed that the ascorbic acid content of the various milk samples varied with the cow almost as much as it varied with the breed, and that the ascorbic acid content of milk from some cows varied from period to period. In this work they called attention to the apparent relation between cardboard flavor and low ascorbic acid content although they evidently did not consider it important, since it was not included in their summary. They stated that invariably the samples which developed cardboard flavor on standing, decreased in ascorbic acid content coincident with the development of the off flavor. They did not determine whether the cardboard flavor was due to the degradation products of ascorbic acid or whether the factors instrumental in the formation of cardboard flavor were also instrumental in the destruction of ascorbic acid.

Ross (35) presented data showing that under commercial conditions of pasteurization the destruction of vitamin C was due principally to the presence of copper in the apparatus used. Furthermore, copper contamination from a bottle filler increased the oxidized flavor and decreased the ascorbic acid (vitamin C) in pasteurized milk.

Sharp (36) stated that in agreement with the work of Whitnah and Riddell (47) the vitamin C content of fresh milk was found to be relatively constant throughout the year although variations occurred in individual cows. He stated that no increase in the ascorbic acid content of cow's milk was produced by green feeding nor of goats milk by intra-jugular injection of four grams ascorbic acid daily. He explained the variations in the rate of oxidation of ascorbic acid in milk by assuming the presence of an ascorbic acid oxidase, the action of which was highly catalyzed by traces of dissolved copper and by individual cows which gave milk of varying ascorbic acid oxidase activity. Certain experiments indicated that ascorbic acid disappeared more rapidly from winter milk produced on dry feed than it did from summer milk. This he attributed to different amounts of enzyme or of copper at these seasons. He found the holder method of pasteurization (30 minutes at 143° - 145°) caused a slight destruction of the enzyme while a half minute or longer of high temperature pasteurization (77°C. or 170°F.) destroyed the enzyme entirely and so retarded oxidation of the ascorbic acid. Even traces of copper dissolved in the milk pasteurized by the latter method had very little acceleration effect upon the destruction of ascorbic acid because the enzyme had been killed by the high temperatures. He pointed out that the idea that raw milk contained more vitamin C than holder pasteurized milk has been a false conception since he found that milk heated in glass for one hour at 63°C. contained more ascorbic acid after holding cold three days than did an aliquot held raw for the same time. This he stated was because more of the enzyme, oxidase, which catalyzed the oxidation of ascorbic acid was destroyed by heating than was the ascorbic acid itself. He attributed any appreciable destruction of vitamin C in commercial pasteurization to copper contamination, and s stated that most of the destruction of ascorbic acid in commercial bottled milk occurred in the cold milk during holding after pasteurization.

Prewitt (28) had likewise noted that as the pasteurization temperature was increased with a shorter period of holding the intensity of oxidized flavor was correspondingly lessened, and at 93°C. inhibited.

Dann and Satterfield (9); Whitnah and Riddell (48), and Barron, Barron, and Klemperer (2) have discovered some interesting relationships between the ascorbic acid content of milk and certain of its physical and chemical properties but did not correlate their work with the oxidized flavor, however, this work may be considered to have an indirect bearing on the oxidized flavor studies since they studied certain processes which have been shown by others to be involved in the development of oxidized flavor.

Enzymes and the oxidized flavor. Duthrie (14) suggested as early as 1916 that enzymic action was possibly the cause of the "metallic" flavor which he was then studying. This flavor was quite likely identical with that now known as oxidized. Later Kende (22) showed that an enzyme, oleanase, in the presence of copper was the cause of oxidized flavor. He found that the agents responsible for the oxidation were in no way associated with the fats, but were to be found in the milk plasma, particularly in the albumen-globulin fraction of the milk.

Perlman (26) in 1935 published work to show that certain bacteria were capable of producing enzymes which affected the destruction of milk phospholipids, which were found by Brown, Thurston, and Dustman (5), later by Chilson(8), to be oxidized, in part at least, yielding the flavor in question.

Chilson (8) found that the addition of small amounts of copper sulphate to milk which apparently would not oxidize because of the lack of susceptibility, or of enzyme, did become oxidized. This showed that the enzyme alone was not a sufficient catalyst, but had to be aided by the copper.

Gondos (13) attributed the development of the oxidized flavor in part to the presence of oleinase in the fat, and in part to metal contamination which catalyzed the activity of the oleinase.

Chilson (8) pointed out that there was no enzyme present in the milk of some cows during the summer months, thus accounting for the absence of the oxidized flavor during certain seasons of the year.

Sharp, Trout and Guthrie (37) indicated that the enzyme was foremost in importance as a source of oxidized flavor.

<u>Effect of feed on oxidized flavor</u>. Not until 1928 was feed suggested as a factor associated with the oxidized flavor development. Frazier (11) stated that the oxidized flavor due to light developed more rapidly in milk from cows fed heavy rations of cottonseed meal or linseed cake than in milk from cows receiving no oil feed.

Kende (27) expressed the opinion that one factor in the production of the "oleaginous" flavor (oxidized flavor) was the condition and type of feed which the cow received. Gondos (13) expressed the belief that the choice of the proper ration was the key to control of certain reducing substances in dairy products which acted as natural protective agents against oxidized flavor.

Henderson and Roadhouse (18) stated that milk produced by animals drawing upon their body fat as the result of submaintenance rations showed increases in the percentage of unsaturated fats, and showed, likewise, an increased susceptibility of the fat to oxidation.

On the other hand, Chilson (8) stated that the feed did not appear to be a major cause for the seasonal variation in oxidized flavor, as some of the cows in his experiment which gave a very distinct flavor in the winter or spring months, but gave no off flavor in August, were not on pasture, and were getting approximately the same ration which they received in the winter.

Prewitt (28) found that the fat level in the ration influenced the development of oxidized flavor and that certain grain mixtures had an inhibitory effect, but that the vitamin A level in the ration caused no difference in the intensity of the oxidized flavor. However, Anderson, Hardenberg, and Wilson (1) found that the addition of 500,000 U.S.P. units of vitamin A was far less effective than the addition of approximately eight pounds of carrots daily to the ration. They suggested that off flavors in raw milk might be due to the feeding of rations in which certain labile accessory food factors had been greatly diminished by improper curing or by storage. For example, artificially dried alfalfa hay was considerably richer in this quality than field dried hay. Also, carrots were much more potent in the factor than the artificially dried hay. Oxidized flavors were eliminated from the milk by addition of carrots to the ration. They believed that carotene alone or with associated compounds was concerned with the prevention of oxidized flavors in raw milk.

Prewitt and Parfitt (29) indicated that both the feed and the individuality of the cow were factors which governed the susceptibility of the milk to oxidized flavor development. They postulated that certain feeds, particularly green feed, impart an antioxidant to the milk, which account, in part, for the lessened tendency for oxidized flavor development in summer.

Workers at the West Virginia Agricultural Experiment Station (10) found that the feeding of tomato juice, lemon juice, pasture, or pure vitamin C (cebione) prevented the development of the oxidized milk. They attributed this inhibitory effect to the vitamin C present in the feed.

Brown, Thurston, and Dustman (6) found that changing cows on dry feed to a dry feed plus pasture regime caused milk to become non-susceptible to oxidized flavor development. Milk from individual cows was found to vary in susceptibility to oxidized flavor development. Their work indicated that the feed of the cow had a very pronounced effect on the susceptibility of the milk to oxidized flavor development. Tomato juice, lemon juice and ascorbic acid fed to the cows all had a pronounced effect in reducing the susceptibility of the milk to oxidized flavor development.

Effect of freezing and thawing of milk on oxidized flavor. Recently Thurston, Brown and Dustman (42) called attention to the observation that freezing and thawing caused the reduction or elimination of the susceptibility of milk to the oxidized flavor development. They noted a close relationship between this effect and the occurrence of cream plugs containing relatively large particles of butterfat, that presumably oiled off during thawing. Whenever an excessive cream plug was observed, the milk failed to develop oxidized flavor even with 3.9 parts per million of added copper. However, when a less pronounced cream plug was observed some development of oxidized flavor usually was evident.

Effect of heat on oxidized flavor development in milk. For some time it has been known that milk susceptible to oxidized flavor and contaminated with copper and other metals develops a pronounced oxidized flavor upon holder pasteurization. Kende (22) found, in addition to the effects of holder pasteurization, that high temperature pasteurization (80° C. for five minutes) would inhibit the development of oxidized flavor even in the presence of heavy metal contamination.

Guthrie and Bruecner (15) noted no difference in the intensity of the oxidized flavor developed when milk was pasteurized at temperatures between 140° F. and 150° F. for thirty minutes, but that in milk pasteurized at 160° F. oxidized flavor developed although its intensity was diminished. Pasteurization at 170° F. yielded a cooked flavor but oxidized flavor did not develop upon storage.

Perlman (27) found no evidence that heat alone caused the destruction of lecithin and its allied phospholipids, which have been shown (8, 41) to be associated with oxidized flavor. He showed that certain bacteria, however, were capable of producing enzymes which would effect the destruction of milk phospholipids.

Prewitt (28) showed that as pasteurization temperature was increased with a shorter period of holding the degree of oxidized flavor was correspondingly lessened until at 93°C. its development was inhibited. He attributed this to reduction of time of contact with the copper rather than the higher pasteurization temperature.

Effect of homogenization on oxidized flavor in milk. In 1933 Tracy, Ramsey, and Ruehe (44) discovered that homogenization of milk contaminated with copper caused the tallowy flavor in milk to be less apparent. However, homogenization had no apparent effect on oxidation-reduction potentials of the milk. They also found that the more gelatin they added the less apparent the flavor became. They attributed the lessening in intensity of flavor to some difference in the taste reaction rather than any actual reduction in the intensity of the oxidation of the butterfat. They stated that the close relationship between Eh values of the homogenized and the unhomogenized milk indicated that the lessened intensity of the oxidized flavor developing in the homogenized milk needed to be explained in some other way than on an oxidation-reduction basis.

Thurston, Brown and Dustman (42) found that unhomogenized milk containing no added copper failed to develop an oxidized flavor; that unhomogenized milk copper contaminated from the cylinder block and valves of the machine became oxidized when processed without pressure; that 500 pounds pressure reduced the oxidized flavor development materially or destroyed it completely; and that oxidized flavor did not develop in milk containing added copper when the homogenization pressure was 3000 pounds or more.

Ross (35) observed that pasteurized milk which contained copper and which was homogenized at 1500 pounds pressure did not develop an oxidized flavor whereas lower pressures did not prevent this flavor development.

<u>Effect of the individual cow on oxidized flavor in milk</u>. Henderson and Roadhouse (18) noted that milk produced by animals drawing upon their body fat by the consumption of submaintenance rations showed increases in the percentage of unsaturated fats and increased susceptibility of the fat to oxidation.

Guthrie and Brueckner (15) found that of 155 cows studied 21 per cent gave milk which distinctly developed oxidized flavor, and an additional 10 per cent gave milk which developed slightly oxidized flavor. They stated that apparently the oxidized flavor persisted in the milk of certain cows even through its appearance for the most part was irregular.

Trebler (45) stated that animals might be a cause of oxidized flavor as well as plant equipment.

Chilson (8) noted variations of oxidized flavor in the milk from different cows. He found that the milk from some cows developed the flavor more distinctly than that from others. He noted also that with some cows this flavor development persisted for several weeks or months, and then disappeared.

Later he (8) studied the vitamin C content of milk from different cows some of which were on pasture and some of which were on dry feed. Practically all of these cows gave milk which developed the oxidized flavor in the winter months, but none gave the flavor in August.

Prewitt (28) recorded an instance in which a certain cow gave positive mastitis reaction to the brom cresol purple paper test on four different occasions and, although her milk was not judged the best, at no time was it scored low because of the presence of a metallic or oxidized flavor. He called attention to the fact that Horrall (21) had shown that the per cent of lecithin in fat of milk from mastitis cows ranged from 0.348 to 5.32 with most of the samples above 0.9. In the fat of milk from healthy udders the range was 0.507 per cent to 0.743 per cent. Prewitt believed that in view of Horrall's findings lecithin would appear not to be a source of oxidized flavor. Likewise, milk from a cow which was suffering from mastitis was not more likely to develop an oxidized flavor than the milk from a normal cow.

Prewitt and Parfitt (29) found the individuality of the cow to be a factor which governed the susceptibility of the milk to oxidized flavor development. Whitnah and Riddell (48) showed that the season of the year, the individuality of the animal, the breed of the cow, and the stage of lactation appeared to be the most important factors causing variations in the vitamin C content of fresh milk, which is thought by several (8, 37, 30, 35) to be directly connected with the development of oxidized flavor.

Effect of light on oxidized flavor development in milk. Hammer and Cordes (17) found that sunlight had a pronounced influence on the flavor of milk and cream. They found that with sufficient exposure a definitely oxidized flavor developed and that with less exposure a distinctly "off" flavor occurred. They were able to prevent this flavor by using brown glass bottles.

Frazier (11) became interested in a defect in milk during cold weather which was bothering people who were using "outdoor ice boxes" of the open window-box type. The milk developed a "cardboard" flavor and linseed oil odor. He found this flavor to develop in whole milk exposed to diffuse daylight for eight or more hours even at about freezing temperatures. The defect developed faster in pasteurized than in raw milk.

Guthrie and Brueckner (15) found that ultra violet light, acting as a catalyst, was the cause of some of the oxidized flavor development in milk.

Henderson and Roadhouse (18), in their study of susceptibility of milk to the oxidized flavor by use of the photochemical cell, found that exposure of cream to the action of direct sunlight or to diffused light gave definite increases in susceptibility.

<u>Oxidized flavor as a result of metal contamination</u>. From the knowledge of the fact that oxidized flavor is the result of the oxidation of the fat or some of its constituents rather than the hydrolysis of the fat, the catalyzing effect of metal in the oxidation process has been pointed out by a number of investigators.

Golding and Feilmann (12) were the first to correlate the oxidized flavor with copper contamination. They found that when milk was cooled over a little farm cooker which had the greater part of the tinning removed, allowing bare copper to come into contact with the milk, a distinct flavor developed in about eighteen hours at room temperature. The milk was found to contain two and one-half parts of copper in 10,000,000 parts of sweet milk. They stated "the chemistry of the flavor is still only a matter of speculation, but similar flavors can be produced by other oxidizing agents such as potassium permanganate, ferrous chloride and hydrogen peroxide." They also thought micro-organisms might be the fundamental cause.

Guthrie (14) concluded that direct absorption of metals might cause the metallic flavor in dairy products.

In connection with the theory of metal contamination and the oxidized flavor development in milk it is well to review the work of Miscall, Cavanaugh and Carodemas (24) who showed that the solubility of copper in pasteurized milk increased up to a temperature of 140°F. and then decreased as the temperature was raised. Both increase and decrease in solubility were shown to have taken place gradually. In raw milk the same type of curve was shown except the temperature of maximum copper solubility was 150°F. They also noted that previously pasteurized milk dissolved more copper at every temperature than did the raw milk. Milk under partial vacuum had a much lower copper solubility than either the raw or pasteurized milk. The maximum copper solubility in this case was at 140°F. Carbon dioxide was shown to have a definite depressing effect upon the solubility of copper in milk. When oxygen was used instead of carbon dioxide the maximum dissolving power was exhibited.

Guthrie, Roadhouse, and Richardson (16) suggested that corrosion of metals by milk is truly electro-chemical and agreed with Rice and Miscall (31) that copper once dissolved in milk may plate out on any tin surface with which it comes into contact. Also, that whenever milk is exposed to surfaces of tin and copper together, less copper is dissolved than where there is the same exposure of copper alone.

Guthrie and Brueckner (15) in reference to the oxidized flavor stated that so far as was known oxidation of milk fat in milk and cream is the cause of these flavors and that copper, acting as a catalyst, is largely responsible for the development of the flavor.

Trebler (45) indicated that copper, iron or zinc will cause, or rather accentuate, the oxidized off flavor in milk.

Tracy, Ramsey, and Ruehe (44) found that upon innoculation of milk with copper the oxidation-reduction potential was found to move toward the side of oxidation. Ritter and Christen (33) found that tallowiness in milk was associated with 0.01 milligrams of copper per liter; five milligrams per liter also produced the flavor. Adding hydrogen peroxide, hydrogen ions, or N-methyl-p-amidophenol-sulfate stopped the development of tallowiness. Ascorbic acid and molic acid had a like action. Increasing temperatures of heating decreased the tendency for this flavor to develop.

Kende (22) produced the "oleigenous" flavor by exposing milk to metal squares, a ten cm square to 500 cc milk. He found that salts of the metals were far superior in producing the flavor, also that 1/10 the amount of copper would cause the same effect as iron.

Gondos (13) stated that contamination of dairy products with heavy metals which catalyze the activity of the oleinase is among the factors which operate to cause oxidized flavor.

In 1934 Henderson and Roadhouse (18) showed that exposure of cream to copper definitely increased its susceptibility to oxidized flavor.

Thurston, Brown and Dustman (41) demonstrated that pasteurization of milk to which 0.01 per cent powdered copper has been added produced the oxidized flavor in milk when it was cooled to 40°F. and air bubbled through it for 24 hours. This treatment produced a strong oxidized flavor while the same treatment without the added copper produced no oxidized flavor.

Brown, Thurston, and Dustman (5) stated that when milk was contaminated with an amount of copper ranging up to two and one-half ppm after being holder pasteurized, developed a more intense oxidized flavor than when identical contamination took place before pasteurization. Raw milk contaminated with copper had a tendency to develop the same intensity of oxidized flavor as the same milk contaminated after pasteurization. The exposure of milk to the air while being passed over a surface cooler had no more tendency to produce oxidized flavor than the passage of the milk through an internal tube cooler.

The oxidation of fatty constituents of milk as a cause of Oxidized flavor. A survey of the literature on this subject reveals many conflicting data relative to which particular fatty constituent yield the oxidized flavor. Thurston and Barnhart (40) believed that lecithin was connected with the oxidized flavor. They made their studies on buttermilk "because it contained the 'hull' from the fat globules in churned cream." They washed the "hull" from some of the fat globules and allowed it to remain on others. They made synthetic milk and added lecithin to it and also added lecithin to skimmilk in which the per cent lecithin was known. They found that samples that contained lecithin developed oxidized flavor while those containing none and those with washed fat globules produced no oxidized flavor.

Thurston, Brown and Dustman (41) heated milk to 65°F. to 70°F. and separated it. They then standardized the cream to 35 per cent and divided it into two lots. One lot was churned in glass; the other lot was converted into washed butterfat. This washing removed all phospholipids since no phosphorus could be found in the washed butterfat. They redispersed the washed butterfat in skim milk of good flavor by homogenization. The remade milk did not develop oxidized flavor. From their findings they concluded that lecithin rather than butterfat appeared to be the constituent in butterfat affected when oxidized flavor developed and that there were indications that the so called oxidized flavor was not identical with the tallowy flavor of oxidized butterfat. Recent investigation, however, has shown that homogenization will in itself prevent the development of oxidized flavor in milk. In this work the milk was remade by homogenization.

Chilson (8) supported their theory stating, "The oxidized flavor is never found in the skim milk unless the fat content is too high. This shows that the oxidized flavor due to oxidation of the fat or substances adsorbed on the fat globule."

Later Chilson (8) stated that milk which was susceptible to the oxidized flavor was separated and the fat washed 14 times and then mixed back with its original skim milk. The remade milk produced no oxidized flavor even when copper sulfate was added. This evidence he said seems to show that it is not the actual milk fat which is oxidized but something adsorbed or at least something that can be washed off from the fat globule such as lecithin.

<u>Lecithin values in dairy products</u>. To understand the theory

of oxidized flavor due to oxidation of the lecithin, or perhaps more strictly speaking, to the oxidation of the fatty constituents adsorbed on the surface of the fat globule, it is necessary first to become familiar with the results which come from much speculation and investigation on the subject of lecithin as a cause of error in the various fat tests used in the dairy industry. Chapman (7) was one of the first to study this error. Thurston and Peterson (43) shortly afterward reported indications that lecithin content materially altered the fat test when certain methods were used. Holm and his associates (19, 50, 51) studied the phospholipid content of various dairy products with particular interest in the error of certain fat tests due to phospholipid materials.

Palmer and Weise (25) studied the materials adsorbed on the fat globule and reported that they had found, by dialysing the "membrane" of fat globules from churned washed fat in collodian bags, that there was no casein adsorbed there. However, they found that phosphorus which others had claimed an impurity was not dialysed off, and hence was a definite part of the material adsorbed on the surface of the fat globule. Soon after, Perlman (23) showed that the phospholipid content of fresh cream increased uniformly with the fat content to a point of approximately 55-58 per cent milk fat (the point at which evidently a reversion of the colloidal system took place) after which it decreased with further fat increases. He found no indications that heat alone would affect the destruction of phospholipids in milk products.

Kurtz, Jamison, and Holm (23) analysed the fatty acids of the lecithin-cephalin (ether extract) fraction of sweet cream spray dried buttermilk powder. They found that the ether soluble material was made up of 35 per cent cephalin and 65 per cent lecithin and that in contrast to butterfat the lecithin-cephalin fraction of the milk phospholipids contained none of the lower fatty acids. More surprising was the entire absence of palmitic acid so widely distributed throughout most oils and fats of either vegetable or animal origin. The high percentage of oleic acid found shows that a considerable portion of the phospholipid molecules contained only unsaturated acids.

Horrall (21) made an extensive study of the lecithin content of various dairy products. He indicated the lecithin content of milk from a normal cow to be fairly constant after the first four days of the lactation period. Factory milk was shown to be much higher in lecithin content than milk from a healthy cow. Mastitis cows were shown to give a high per cent lecithin milk.

All of these findings by the various investigators just referred to have a direct bearing on the possibility of lecithin being the substance that is oxidized thus producing the oxidized flavor in milk.

Of particular importance to anyone studying the relationship of lecithin to the oxidized flavor in milk is the work of Holm, Wright and Deysher (20) who published work on the chemical nature of the phospholipids in milk. They found that 60.43 per cent of the total isolated material was lecithin; 32.37 per cent of it was cephalin and 7.20 per cent was sphingomyelin.

The average molecular weight of the lecithin-cephalin fraction was calculated to be 775.6 on the basis of 8.4 parts lecithin to each 4.5 parts of cephalin. They state that unpublished data they had already collected showed the sphingomyelin-cerebroside factor to indicate the molecular weight of sphingomyelin as 805. The distribution of the lecithin-cephalin to sphingomyelin was 12.9 to 1.0. On this basis the average molecular weight of the phospholipid material would be 775.76 or slightly higher than if it were a lecithin of the oleo-stearyl or distearyl type. Phosphorus represents four per cent of this weight and hence the lecithin factor for conversion of phosphorus to milligrams of phospholipids would be 25.0.

They stated that in view of the solubility of these different fractions it is doubtful if extraction of phospholipids can be complete without the use of hot ethanol or the supplementary use of extraction with chloroform. The following is a table which they give showing the phospholipid content of milk and milk products:

Phospholipid Content of Milk and Milk Products According to Mohr, Brockman and Muller, Horrall and the Authors

Product	Mohr, Brockman and Muller per cent	Horrall per cent	Holm, Wright and Deysher per cent
Whole Milk	0.037	0.0276	0.0337
Skim Milk	0.0155	0.0166	0.0169
Cream	0.1685 (23%)*	0.155 (37.87%)*	0.1318
Butter	0.2060	0.1685	0.1819
Buttermilk	0.1142	0.1415	0.1872
Separator Slime	--	0.68	--

* per cent fat in the cream.

<u>The effect of oxidation-reduction potentials on oxidized flavor</u>. Thornton and Hastings (39) defined oxidation as the process in which a substance takes up positive, or parts with negative charges, while reduction is the process in which a substance takes up negative or parts with positive charges. They stated that these electronic charges can be followed in milk potentiometrically. The positive Eh values of all milks which they studied lay between +0.2 and +0.3 volts. Negative potential limits reached by all milks reported approximately Eh -0.2 volts due, it was suggested, to the predominating influence of S. Lactis.

Tracy Ramsey and Ruehe (44) stated that oxidation-reduction potentials showed the normal tendency toward reduction in freshly drawn milk. Upon the introduction of copper the potential was found to be more toward the side of oxidation. Incubation of the milk usually caused a rapid drop in potential. They pointed out that oxidation-reduction potentials were related to fat oxidation in dairy products. Bacteria and yeast results in a change of potential toward the reduction phase, which suggests a removal of oxygen occurs through the metabolism process of the organisms. This undoubtedly explains why milk of very good quality, from a bacterial standpoint, is more likely to become tallowy than in milk more highly contaminated, they pointed out. They found than an Eh value of 0.3000 was the approximate point above which oxidized flavors were likely to occur. They also found that the oxidation-reduction potential was apparently not affected when milk was homogenized although homogenization caused the tallowy flavor to be less apparent.

Sharp, Trout, and Guthrie (39) in their study of ascorbic acid destruction in milk and the relationship it played in the development of oxidized flavor found that the control of the oxidation-reduction potential of milk was a very important factor. They collected evidence indicating that the more rapidly the ascorbic acid oxidized the more rapidly the oxidation potential, hence, would account for more rapid development of the oxidized flavor, they pointed out.

Webb and Hileman (46) reported that the oxidation-reduction potential offered a very reasonable explanation of the effect that copper has in catalyzing oxidized flavor development, and that apparently copper produced the flavor in winter by raising the potential to a point sufficiently high to cause some milk constituent to be affected. In the summer season this same potential was not high enough to produce the flavor change. They stated that there was no relationship between oxidation-reduction potentials of milk of individual cows and the oxidized flavor. Oxidized flavors in such samples occasionally developed when the oxidation-reduction potentials were quite low.

The effect of agitation of milk on oxidized flavor. Thurston, Brown and Dustman (42) called attention to the transfer of lecithin from adsorbed layers on the fat globule to the plasma of milk caused by vigorous agitation of cold milk. Agitation caused a reduction in the lecithin content of the skim milk. Vigorous agitation caused susceptible milks to be less susceptible or to be nonsusceptible. Extensive changes at the fat globule surfaces were indicated by the fact that the agitation of raw milk caused the subsequent development of rancid flavor in every case. Cream plugs, formed on all the pasteurized milk, also indicated that changes at the fat globule surface occurred as the result of agitation.

PROCEDURE

The milk used in the greater part of this experiment was secured from individual animals of a group of eight cows containing two Brown Swiss, one Guernsey, two Holsteins and three Jerseys. These animals had been selected for a double feed reversal experiment in which the level of the fat in the ration was to be the variant. One-half of the cows were placed on a high fat ration while the other half were placed on a similar ration, but of low fat content. The cows received a basal ration of good quality alfalfa hay, beet pulp, solvent extracted soy bean flakes, bone meal, and molasses. The high fat group of cows received soy bean oil in proportion to the fat content of the milk while the low fat group received no fat other than that present in the above ration. After three reversals of the high and lot fat groups, the final low fat group was placed on a ration of ground corn and alfalfa hay for several days, during which time samples were secured. Samples of milk were taken every fourth day throughout the entire period except at the change over period when extra samples were taken on the second day. Later, additional samples of milk were taken from a special group of four cows, consisting of one animal from each of the four major dairy breeds. Samples from this group were studied while the cows were on a regular balanced dairy ration and after pasture had been added to their ration.

All of the cows were milked by hand into sterilized porcelain pails throughout the entire experiment. After weighing, the milk was mixed by pouring back and forth from one pail to another four or five times. Samples were taken immediately by pouring directly into a regular quart milk bottle. The bottles were corked, using large sterilized corks rather than bottle caps, after which they were set in a bucket of cold water to cool. When all of the samples had been taken they were transported immediately to the laboratory where they were further cooled in ice water. After cooling, each sample was mixed thoroughly and a 500 ml portion taken for pasteurization in another quart milk bottle. To this sample was added .5 ml of a solution containing 2.5 grams copper per liter in the form of copper sulphate to produce a concentration of2.5 ppm of copper in the milk. The milk was then pasteurized in glass at 143°F. for 30 minutes and cooled. Immediately after cooling the samples were tasted for oxidized flavor development. They were then placed in an electric refrigerator at 38°F. for holding. After 24, 48, 72 and 96 hours storage the milk was removed from the refrigerator and warmed to room temperature, after which the flavor was determined organoleptically. The tasting was done by the writer and one or more other experienced flavor judges.

The symbols which were used to designate the different intensities of oxidized flavor were as follows:

-, no oxidized flavor

?, questionable (this group was judged by experienced judges but would not be noticed by the ordinary consumer)

+, distinctly oxidized, yet not objectionable

++, badly oxidized

+++, very badly oxidized.

Oxidation-reduction potentials (Eh determinations) were run in duplicate on the original samples of fresh raw milk using a bright foil platinum electrode and a saturated colomel half-cell. The EMF readings were taken from a portable Leeds and Northrup potentiometer. The saturated colomel half-cell was used instead of a normal colomel half-cell because of its greater stability. The bright foil platinum electrodes were kept emmersed in distilled water whenever they were not in use and the milk was rinsed from them with slightly warm water between each sample. Whenever the electrodes showed evidence of poisoning they were dipped into a dilute solution of hydrochloric acid.

The EMF readings were converted to Eh by adding to them the voltage of the saturated colomel half-cell for the temperature at which the readings were made since Eh is based upon the use of a normal colomel half-cell and a hydrogen electrode. The potential for a normal ~~colomel half-cell against a~~ hydrogen electrode is zero volts, thus making it necessary to use a correction factor when the saturated half-cell and platinum electrodes are used. The voltage of the saturated colomel half-cell used was +.2432 volts at 25°C.

The determination of phospholipids in milk was accomplished in two steps. First, the fatty materials were extracted and then the organic phosphorus determined by a colorimetric method.

The fatty materials were extracted by the method followed by Worrall (21) with the exception that rather than pipetting a ten-gram sample of milk into the extraction flask approximately a twenty-gram sample was weighed into two of the extraction flasks. Duplicates were run on each sample of milk. The fat was dried in the regular manner and weighed back, after which the per cent fat was calculated. If the duplicates did not check within two or three one-hundredths of one per cent the sample was rerun.

Because of difficulty in obtaining clear blanks for phosphorous on reagents used in the above procedure, purification of all reagents used in the extraction was found necessary. Accordingly, phosphorus and arsenic free distilled water was secured; a special ammonia hydroxide very low in arsenic and phosphorus was obtained; and the ethers and alcohol redistilled. The ethyl ether was redistilled over metallic sodium; the petroleum ether was redistilled over alcoholic potassium hydroxide; and alcohol was redistilled over potassium hydroxide after being refluxed for one hour over aluminum turnings according to the method of Stout and Schuette (38).

After the fat pans were weighed back they were returned to the edge of the fat plate where the fat was again liquified. The fat was then carefully washed into a seven cm Sillimanite crucible by means of a glass rod and a solution of alcohol, petroleum and ethyl ether mixed in the same proportions as that used in the extraction. Careful checking on the fat weights showed no loss through this transfer, although it required some three or four washings before all of the fat was removed from the pan. This necessitated filling the ashing crucibles quite full, but no particular difficulty was encountered. The ether-alcohol solution was allowed to evaporate from the fat by standing at room temperature over night. Then a saturated solution of alcoholic magnesium nitrate was pipetted into them for ashing at the rate of one-half cc for each 0.1 gram of fat. The crucibles were then placed on the Mojonnier hot plates and, by means of the rheostatic controls, the temperature was gradually raised from 90°F. to 180°F. Rotating of the crucibles in such a manner as to mix the contents greatly aided in the drying process. With proper care in increasing the temperature and in numerous agitations of the contents of the crucible all sputtering was eliminated. As soon as the samples were dry they were placed in an electric muffle oven at red heat and allowed to remain there until a white ash remained. After cooling, sufficient hot ten normal sulfuric acid was added from a wash bottle to cover the ash. If this did not dissolve the ash, along with a little rotating of the crucible, boiling hot distilled water was added and the dissolving completed by rotating. The dissolved ash was washed from the crucible into a 200 ml blue line volumetric flask by the use of boiling hot water and a clear rubber policeman. Checking of the crucibles for phosphorus disclosed that as much as six washings and liberal use of the policeman were necessary to remove all of the phosphorus from the crucible. Accordingly, all crucibles were washed at least seven or eight times and the wash water decanted into the flask before the crucible was set aside. The sample was then neutralized with cp ammonia water using phenolphthalein as indicator, and the volume made to 200 ml with arsenic and phosphorus free distilled water. From the 200 ml of solution, 10 ml was carefully pipetted into a 100 ml volumetric flask. To this flask the colorimetric reagents for phosphorus were added and the volume made up to 100 ml. Solution from this flask was poured into the cup of a Basuch and Lomb Biological Colorimeter with the standard set at 20. The phosphorus was determined by the colorimetric method of Bodansky (4) with the exception that the standard phosphate solution was made up according to the method used by Horrall (21) and his method of converting the reading to milligrams of phosphorus was followed. The factor 25.94 was used for converting mg of phosphorus to mg of lecithin since the newer figure of Holm, Wright, and Deysher (20) was not published at the time the conversions were figured.

The correlations presented in this thesis were calculated after the method outlined by Arkin and Colton (2).

The data from which these correlations were figured were those obtained in the analysis of the various samples of milk studied with no regard taken for the ration upon which each sample was produced.

EXPERIMENTAL

<u>Effect of high and low fat rations on lecithin content of milk.</u> In this series of experiments access was had to two groups of four cows which were being fed high and low fat rations in a double reversal feeding experiment. These two groups were made up of two Brown Swiss, one Guernsey, two Holsteins and three Jerseys.

The two groups of cows were started, one on a low fat ration, the other on a high fat ration. The only difference in the two rations as set forth in the procedure, was that the high fat group received soy bean oil according to the fat in the milk in addition to the basal low fat ration. After 24 days the two groups of cows were reversed, the low fat group receiving the oil this time and the high fat group receiving none. Samples were taken at the mid-day milking every fourth day during the period except at the change-over when an extra sample was taken on the second day after reversal. Two reversals were made, thus extending the experiment over three periods of 24 days each. The samples were aliquoted and analyzed for lecithin; the oxidation reduction potentials determined; and the oxidized flavor studied according to the procedure already outlined. The data secured was presented in Tables 1 to 8 inclusive. The figures recorded are the average of carefully analyzed duplicates except in the case of flavor samples where duplicates were considered unnecessary.

Table 1. Effect of the Fat Level in the Ration upon the Lecithin Content of the Milk and Upon the Susceptibility of the Milk to the Development of the Oxidized Flavor.
(Cow No. 7)

Nature of Ration	Date of sample (1936)	Wt. of noon milk (lbs)	Fat test (%)	Lecithin in milk (%)	Lecithin in fat (%)	Oxidized* flavor score	Eh value in volts
High fat	Jan. 17	10.4	6.288	0.05996	0.9536	?	0.3043
"	" 21	11.0	6.227	0.04763	0.7729	?	0.3144
"	" 25	8.2	6.443	0.04853	0.7525	?	0.3379
"	" 29	8.6	5.747	0.06014	1.0963	-	0.3296
"	Feb. 2	11.1	5.044	0.04411	0.8745	++	0.3177
"	" 6	12.0	4.746	0.05160	1.0862	+++	0.2989
Low fat	" 8	10.7	4.505	0.04687	1.0403	-	0.2991
"	" 10	8.6	5.619	0.05065	0.9014	?	0.2917
"	" 14	7.8	4.675	0.05257	1.1242	?	0.2192
"	" 18	7.3	5.733	0.05697	1.2078	?	0.2273
"	" 22	7.0	7.472	0.05468	0.7317	-	0.1976
"	" 26	6.4	5.753	0.05453	0.9478	?	0.2510
"	Mar. 1	5.9	5.565	0.04563	0.7826	-	0.2098
High fat	" 3	6.4	8.134	0.04858	0.5973	?	0.2697
"	" 5	7.9	7.746	0.05307	0.6807	++	0.2783
"	" 9	6.7	7.096	0.05690	0.7945	++	0.2568
"	" 13	7.0	6.947	0.04878	0.7020	+	0.2100
"	" 17	7.0	5.932	0.05079	0.8537	++	0.1982
"	" 21	7.2	6.001	0.04249	0.7080	+++	0.2153
"	" 25	6.6	5.372	0.04107	0.7639	+++	0.2272

*Key -- 48 hours at 38°F:
- No oxidation
? Questionable
\+ Distinctly oxidized flavor
++ Badly oxidized flavor
+++ Very badly oxidized flavor

Table 2. Effect of the Fat Level in the Ration upon the Lecithin Content of the Milk and Upon the Susceptibility of the Milk to the Development of the Oxidized Flavor.
(Cow No. 66)

Nature of Ration	Date of sample (1938)	Wt. of noon milk (lbs)	Fat test (%)	Lecithin in milk (%)	Lecithin in fat (%)	Oxidized* flavor score	Eh value in volts
High fat	Jan. 17	9.9	5.071	0.07859	1.5495	?	0.3039
"	" 21	4.7	6.301	0.06494	1.0298	+	0.3023
"	" 25	6.5	4.433	0.04722	1.0650	?	0.3028
"	" 29	7.8	3.917	0.05819	1.4855	?	0.3211
"	Feb. 2	9.4	2.937	0.03605	1.2133	-	0.3137
"	" 6	6.2	3.837	0.04089	1.0653	?	0.3013
Low fat	" 8	7.3	4.896	0.05353	1.0945	-	0.2732
"	" 10	8.0	5.190	0.05717	1.1014	-	0.2854
"	" 14	7.7	5.025	0.05793	1.1576	+	0.1322
"	" 18	8.0	5.830	0.04975	0.8532	-	0.2122
"	" 22	7.4	6.111	0.05450	0.8916	-	0.2077
"	" 26	6.7	6.143	0.05591	0.9103	-	0.2472
"	Mar. 1	4.7	2.453	0.04057	1.7200	-	0.2674
High fat	" 3	7.8	6.914	0.03889	0.5624	-	0.2688
"	" 5	5.7	6.175	0.04899	0.7934	?	0.2680
"	" 9	7.0	5.531	0.04829	0.8730	+	0.2590
"	" 13	8.0	5.854	0.05036	0.8524	+	0.2082
"	" 17	7.0	4.680	0.03241	0.6925	-	0.1998
"	" 21	8.0	6.193	0.03903	0.6301	-	0.2137
"	" 25	8.0	5.821	0.04391	0.7541	-	0.2264

*Key -- 48 hours at 38° F:
- No oxidation
? Questionable
+ Distinctly oxidized flavor
++ Badly oxidized flavor
+++ Very badly oxidized flavor

Table 3. Effect of the Fat Level in the Ration upon the Lecithin Content of the Milk and upon the Susceptibility of the Milk to the Development of the Oxidized Flavor.
(Cow No. 99)

Nature of Ration	Date of sample (1936)	Wt. of noon milk (lbs)	Fat test (%)	Lecithin in milk (%)	Lecithin in fat (%)	Oxidized* flavor score	Eh value in volts
Low fat	Jan. 17	8.5	7.030	0.02461	0.3501	?	0.3022
"	" 21	7.8	6.329	0.05301	0.8377	+	0.3221
"	" 25	7.8	7.524	0.05135	0.6824	+	0.3177
"	" 29	7.7	7.246	0.04398	0.6154	+	0.3077
"	Feb. 2	9.5	6.810	0.04920	0.7225	++	0.2976
"	" 6	6.3	8.460	0.05216	0.6141	+	0.2887
High fat	" 8	8.4	8.130	0.05075	0.6241	++	0.2343
"	" 10	8.0	7.624	0.05030	0.6598	-	0.2641
"	" 14	7.9	7.371	0.06216	0.8413	+	0.2122
"	" 18	8.7	6.683	0.05600	0.8391	++	0.2305
"	" 22	9.5	5.968	0.04625	0.7750	++	0.1991
"	" 26	10.1	6.076	0.04240	0.6979	++	0.2520
"	Mar. 1	9.4	6.535	0.04363	0.7094	+++	0.2664
Low fat	" 3	10.5	4.629	0.03274	0.7073	+++	0.2636
"	" 5	8.1	3.430	0.03545	1.0336	-	0.2623
"	" 9	7.4	5.0508	0.04786	0.94598	-	0.2459
"	" 13	6.7	5.904	0.05007	0.8479	?	0.2127
"	" 17	5.7	5.618	0.03241	0.6925	?	0.1952
"	" 21	6.0	5.738	0.03882	0.6728	-	0.2013
"	" 25	6.0	6.546	0.04269	0.6667	-	0.2149

*Key -- 48 hours at 38°F:
- No oxidation
? Questionable
+ Distinctly oxidized flavor
++ Badly oxidized flavor
+++ Very badly oxidized flavor

Table 4. Effect of the Fat Level in the Ration upon the Lecithin Content of the Milk and Upon the Susceptibility of the Milk to the Development of the Oxidized Flavor.
(Cow No. 101)

Nature of Ration	Date of sample (1936)	Wt. of noon milk (lbs)	Fat test (%)	Lecithin in milk (%)	Lecithin in fat (%)	Oxidized* flavor score	Eh value in volts
Low fat	Jan. 17	11.3	9.830	0.03094	0.3148	?	0.3024
"	" 21	10.5	8.337	0.05651	0.8927	++	0.3029
"	" 25	8.7	8.232	0.05455	0.6375	?	0.3234
"	" 29	8.0	6.389	0.05713	0.8294	-	0.3132
"	Feb. 2	9.6	10.765	0.0752	0.0752	?	0.2966
"	" 6	8.2	6.053	0.05274	0.8710	-	0.2933
High fat	" 8	10.4	9.049	0.05257	0.5809	?	0.2982
"	" 10	8.7	6.843	0.05187	0.7499	-	0.2943
"	" 14	10.1	7.165	0.05951	0.8307	+++	0.2120
"	" 18	11.1	6.381	0.04795	0.7515	++	0.2305
"	" 22	12.6	7.506	0.04906	0.6535	+++	0.1958
"	" 26	12.5	6.150	0.05255	0.8544	++	0.2520
"	Mar. 1	10.2	5.004	0.03849	0.7690	+	0.2042
Low fat	" 3	13.7	7.331	0.04588	0.6261	++	0.2690
"	" 5	14.0	5.154	0.04082	0.7921	+	0.2066
"	" 9	8.0	3.531	0.04744	1.3431	++	0.2527
"	" 13	11.6	7.749	0.04391	0.5648	++	0.2138
"	" 17	10.7	7.800	0.04371	0.5710	?	0.2057
"	" 21	11.4	9.406	0.05097	0.5419	+	0.2094
"	" 25	9.7	7.048	0.04382	0.6212	-	0.2193

*Key -- 48 hours at 38°F:
- No oxidation
? Questionable
+ Distinctly Oxidized flavor
++ Badly oxidized flavor
+++ Very badly oxidized flavor

Table 5. Effect of the Fat Level in the Ration upon the Lecithin Content of the Milk and Upon the Susceptibility of the Milk to the Development of the Oxidized Flavor. (Cow No. 171)

Nature of Ration	Date of sample (1936)	Wt. of noon milk (lbs)	Fat test (%)	Lecithin in milk (%)	Lecithin in fat (%)	Oxidized* flavor score	Eh value in volts
High fat	Jan. 17	16.7	4.739	0.05842	1.2224	-	0.2714
"	" 21	15.6	4.234	0.06347	1.4467	?	0.2972
"	" 25	12.4	3.615	0.04232	1.1698	-	0.3256
"	" 29	14.0	4.387	0.05205	1.1866	-	0.3119
"	Feb. 2	18.2	4.216	0.04981	1.1816	-	0.2992
"	" 6	13.3	3.961	0.05187	1.3092	-	0.2853
Low fat	" 8	15.1	3.391	0.04366	1.2858	-	0.2619
"	" 10	15.0	3.742	0.04917	1.3139	-	0.2477
"	" 14	12.4	3.908	0.06321	1.1329	?	0.1717
"	" 18	13.2	4.034	0.05106	1.2544	-	0.1335
"	" 22	11.9	3.594	0.04171	1.1605	-	0.2124
"	" 26	13.8	4.012	0.05086	1.2777	-	0.2432
"	Mar. 1	9.7	3.310	0.05312	1.6044	-	0.2679
High fat	" 3	12.6	5.003	0.04150	0.8296	-	0.2548
"	" 5	13.1	3.749	0.04312	1.1499	-	0.2591
"	" 9	12.1	3.844	0.04386	1.1410	-	0.2517
"	" 13	12.9	3.766	0.04186	1.0613	-	0.2143
"	" 17	12.0	3.786	0.03446	0.9099	-	0.1943
"	" 21	11.2	3.516	0.03174	1.0450	-	0.1982
"	" 25	13.7	3.01	0.04333	0.6885	-	0.2170

*Key -- 48 hours at 38°F:
- No oxidation
? Questionable
+ Distinctly oxidized flavor
++ Badly oxidized flavor
+++ Very badly oxidized flavor

Table 6. Effect of the Fat Level in the Ration upon the Lecithin Content of the Milk and Upon the Susceptibility of the Milk to the Development of the Oxidized Flavor.
(Cow No. 219)

Nature of Ration	Date of sample (1938)	Wt. of noon milk (lbs)	Fat test (%)	Lecithin in milk (%)	Lecithin in fat (%)	Oxidized* flavor score	Eh value in volts
Low fat	Jan. 17	12.5	2.947	0.04413	1.4983	+++	0.3090
"	" 21	12.2	3.092	0.04845	1.5781	?	0.3219
"	" 25	12.5	3.185	0.03518	1.1350	?	0.3265
"	" 29	12.0	4.220	0.04272	1.0120	-	0.3238
"	Feb. 2	14.2	3.933	0.04194	1.0663	++	0.3074
"	" 6	17.3	3.629	0.03688	1.0164	?	0.2045
High fat	" 8	12.8	3.491	0.03177	0.9106	?	0.2865
"	" 10	14.8	3.567	0.03907	1.0913	++	0.2928
"	" 14	14.3	3.237	0.05568	1.7042	++	0.1917
"	" 18	14.7	3.390	0.03249	0.9582	+	0.2243
"	" 22	14.0	3.083	0.03465	1.1238	++	0.1943
"	" 26	14.3	2.758	0.04405	1.5967	+++	0.2563
"	Mar. 1	12.6	2.497	0.04409	1.7654	+	0.2352
Low fat	" 3	14.0	2.478	0.03246	1.3096	+++	0.2820
"	" 5	15.3	2.155	0.03952	1.8330	++	0.2064
"	" 9	12.3	2.799	0.04303	1.5036	++	0.2472
"	" 13	14.2	2.883	0.03829	1.3311	+++	0.2119
"	" 17	12.1	3.411	0.03385	0.9923	++	0.2045
"	" 21	11.6	3.539	0.04525	1.2782	+++	0.2088
"	" 25	9.5	3.476	0.03833	1.1026	+++	0.2165

*Key -- 48 hours at 38° F:
- No oxidation
? Questionable
+ Distinctly oxidized flavor
++ Badly oxidized flavor
+++ Very badly oxidized flavor

Table 7. Effect of the Fat Level in the Ration upon the Lecithin Content of the Milk and Upon the Susceptibility of the Milk to the Development of the Oxidized Flavor.
(Cow No. 242)

Nature of Ration	Date of sample (1936)	Wt. of noon milk (lbs)	Fat test (%)	Lecithin in milk (%)	Lecithin in fat (%)	Oxidized* flavor score	Eh value in volts
Low fat	Jan. 17	11.0	3.134	0.04543	1.4503	+	0.3084
"	" 21	8.9	4.595	0.05394	1.226	+	0.3067
"	" 25	9.5	5.290	0.04545	0.8590	+	0.3253
"	" 29	6.8	4.193	0.04565	1.0884	-	0.3147
"	Feb. 2	10.7	2.698	0.02885	1.0449	-	0.3070
"	" 6	9.5	4.946	0.04276	0.8645	+	0.3092
High fat	" 8	9.4	5.001	0.03690	1.0155	?	0.2831
"	" 10	9.0	5.023	0.04498	0.8954	-	0.2893
"	" 14	9.0	4.313	0.04580	1.0615	?	0.2376
"	" 18	10.1	4.093	0.04378	1.0752	?	0.2302
"	" 22	12.3	2.634	0.03530	1.1500	++	0.1899
"	" 26	9.6	3.604	0.05525	1.5332	+	0.2547
"	Mar. 1	11.2	3.792	0.03746	0.9931	?	0.2647
Low fat	" 3	11.2	3.163	0.03397	1.0742	+	0.2579
"	" 5	10.6	3.216	0.04442	1.3811	+	0.2584
"	" 9	8.8	3.054	0.04091	1.3391	?	0.2515
"	" 13	9.6	3.288	0.04769	1.4501	++	0.2103
"	" 17	8.0	3.702	0.03830	1.0334	-	0.2043
"	" 21	8.1	4.227	0.04518	1.0689	+	0.2073
"	" 25	7.0	4.962	0.04396	0.8859	++	0.2147

*Key -- 48 hours at 38°F:
- No oxidation
? Questionable
\+ Distinctly oxidized flavor
++ Badly oxidized flavor
+++ Very badly oxidized flavor

Table 8. Effect of the Fat Level in the Ration upon the Lecithin Content of the Milk and Upon the Susceptibility of the Milk to the Development of the Oxidized Flavor. (Cow No. 246)

Nature of Ration	Date of sample (1936)	Wt. of noon milk (lbs)	Fat test (%)	Lecithin in milk (%)	Lecithin in fat (%)	Oxidized* flavor score	Eh value in volts
High fat	Jan. 17	11.2	4.321	0.06198	1.4330	?	0.2994
"	" 21	11.5	3.945	0.05538	1.3592	?	0.3044
"	" 25	12.7	4.395	0.04447	1.0166	?	0.3265
"	" 29	10.2	4.489	0.04856	1.0801	-	0.3265
"	Feb. 2	14.3	3.930	0.04244	1.0786	?	0.3212
"	" 6	12.3	4.294	0.04908	1.1426	+	0.3055
Low fat	" 8	12.9	3.475	0.04608	1.3260	-	0.3042
"	" 10	11.6	3.632	0.04303	1.1846	-	0.2777
"	" 14	12.7	3.823	0.06029	1.5767	-	0.2904
"	" 18	12.6	4.102	0.03703	0.9041	-	0.2214
"	" 22	9.0	4.479	0.04056	0.9061	+	0.2311
"	" 26	8.4	5.452	0.05293	0.9708	-	0.2069
"	Mar. 1	10.6	4.087	0.04327	1.0586	-	0.2472
High fat	" 3	10.3	5.122	0.04054	0.8345	-	0.2577
"	" 5	13.2	4.210	0.03687	0.8757	+	0.2594
"	" 9	12.5	4.389	0.04658	1.0610	-	0.2581
"	" 13	13.5	4.203	0.04410	1.0489	++	0.2555
"	" 17	13.0	4.521	0.03761	0.8317	?	0.2141
"	" 21	11.5	4.309	0.05030	1.1672	?	0.2042
"	" 25	12.6	3.373	0.04582	1.3582	-	0.2014

*Key -- 48 hours at 38°F:
- No oxidation
? Questionable
+ Distinctly oxidized flavor
++ Badly oxidized flavor
+++ Very badly oxidized flavor

The data indicate considerable fluctuation in the lecithin content of the milk regardless of the date upon which the sample was taken and regardless of the ration which the cow received. From these tables it can be readily seen that little relationship existed between the ration which the cow received and the per cent of lecithin which the milk contained. The average lecithin content when all samples were considered was found to be 0.046 ±0.008 per cent lecithin in milk or 0.97 ±0.2697 per cent in the fat. Milk produced on a high fat ration contained no more lecithin than milk produced on a low fat ration. The lecithin content of the milk from the cows while on either ration was equally subject to fluctuation and a change in ration seemed to have no effect on the lecithin content of the milk. The breed of cow seemed to have little effect upon the lecithin in milk; however, the cows which were known to have mastitis gave milk having a higher percentage of lecithin throughout the experiment than those free from the infection. No effect of the age of the animals upon the lecithin content of the milk was noted.

Effect of corn as the sole source of grain in the ration on the lecithin content of the milk. The cows in the final low fat group of the above experiment were placed on a ration of alfalfa hay and corn alone at the close of the last feeding period. They were allowed to remain on this ration for a number of days in order to determine the effect of corn on the lecithin content of the milk. Number two yellow corn having a fat content of about 2.8 per cent was used. The data secured are presented on Table 9.

The results recorded on these trials show no consistent effect of feeding corn on the lecithin content of the milk. In fact, the lecithin content of the milk showed no marked change when this type of ration was started but were quite similar to the values obtained in the high and low fat feeding trials.

Effect of pasture in addition to the regular ration on the lecithin content of the milk. Later in the spring another group of cows was selected for an experiment to determine what effect pasture feeding following a period of dry feeding would have upon the lecithin content of the milk. This group of cows consisted of one animal from each of the major dairy breeds, namely, Brown Swiss, Guernsey, Holstein, and Jersey. The data obtained are given in Table 10. The lecithin content was found to be practically the same before and after the addition of pasture to the ration.

Table 9. Effect of Corn as the only Source of Grain in the Ration on the Lecithin Content of the Milk and upon the Susceptibility of the Milk to the Development of Oxidized Flavor.

Date of sample (1936)	Wt. of noon milk (lbs)	Fat test (%)	Lecithin in milk (%)	Lecithin in fat (%)	Oxidized flavor score*	Eh value in volts
			COW NO. 99			
Mar. 27	6.0	7.284	0.05121	0.7029	?	0.2075
" 29	6.5	6.717	0.04512	0.6761	?	0.2038
Apr. 2	6.3	6.036	0.03914	0.6206	+	0.2055
" 6	6.4	6.821	0.04345	0.6369	-	0.2036
			COW NO. 101			
Mar. 27	7.0	7.797	0.05851	0.7504	?	0.1957
" 29	8.9	(sample lost at barn)				
Apr. 2	9.6	7.245	0.04146	0.5972	?	0.2011
" 6	7.2	6.857	0.04900	0.7146	?	0.2063
			COW NO. 218			
Mar. 27	12.3	3.445	0.04335	1.2583	+	0.2063
" 29	12.2	3.248	0.03114	0.9514	?	0.2120
Apr. 2	13.6	3.229	0.04271	1.3228	?	0.2158
" 6	12.7	3.179	0.03824	1.1489	+	0.2049
			COW NO. 242			
Mar. 27	8.4	4.398	0.05284	1.2014	+	0.2003
" 29	10.7	4.709	0.03739	0.7942	?	0.2072
Apr. 2	11.1	4.941	0.04200	0.8500	-	0.2101
" 6	10.1	4.616	0.04516	0.9782	-	0.2074

*Key -- 48 hours at 38°F:
- No oxidation
? Questionable
+ Distinctly oxidized flavor
++ Badly oxidized flavor
+++ Very badly oxidized flavor

Table 10. Effect of the Addition of Pasture to the Ration upon the Lecithin Content of Milk and upon the Susceptibility of the Milk to the Development of the Oxidized Flavor.

Nature of Ration	Date of sample (1936)	Wt. of noon milk (lbs)	Fat test (%)	Lecithin in milk (%)	Lecithin in fat (%)	Oxidized flavor score*	Eh value in volts
			COW NO. 25				
Dry feed	May 4	13.1	4.432	0.04315	0.9703	?	0.2376
"	" 8	14.3	4.912	0.03643	0.7417	?	0.2504
"	" 11	14.0	4.730	0.04104	0.8669	+	0.2253
Pasture	" 13	14.2	4.952	0.04315	0.8713	-	0.2200
"	" 17	15.8	4.294	0.04409	1.0290	?	0.2197
"	" 21	18.0	4.797	0.04201	0.8803	-	0.2294
			COW NO. 24				
Dry feed	" 4	8.7	7.768	0.04041	0.5954	?	0.2289
"	" 8	9.7	7.473	0.05304	0.7098	-	0.2515
"	" 11	9.0	7.319	0.05689	0.7771	-	0.2307
Pasture	" 13	10.0	7.395	0.05128	0.6934	+	0.2285
"	" 17	11.0	6.664	0.04666	0.7005	+	0.2193
"	" 21	8.1	7.833	0.05322	0.6831	-	0.2330

*Key -- 48 hours at 38°F:
- No oxidation
? Questionable
+ Distinctly oxidized flavor
++ Badly oxidized flavor
+++ Very badly oxidized flavor

Table 10 Continued

Nature of Ration	Date of sample (1936)	Wt. of noon milk (lbs)	Fat test (%)	Lecithin in milk (%)	Lecithin in fat (%)	Oxidized flavor score*	Eh value in volts
			COW NO. 134				
Dry feed	May 4	13.6	4.072	0.04576	1.1239	-	0.2706
"	" 8	11.0	4.880	0.04959	1.0121	-	0.2560
"	" 11	11.3	3.706	0.03985	1.0753	-	0.2370
Pasture	" 13	12.6	4.451	0.05132	1.1639	-	0.2343
"	" 17	15.3	4.177	0.04144	0.9913	-	0.2263
"	" 21	13.3	3.360	0.04551	1.3545	-	0.2054
			COW NO. 193				
Dry feed	May 4	23.4	2.797	0.03023	1.0907	-	0.2722
"	" 8	12.2	2.139	0.03429	1.6026	?	0.2598
"	" 11	18.7	3.367	0.03781	1.1231	-	0.2437
Pasture	" 13	16.0	3.098	0.03889	1.2552	-	0.2328
"	" 17	17.0	3.315	0.03715	1.1948	-	0.2307
"	" 21	14.2	3.083	0.03875	1.2567	-	0.2270

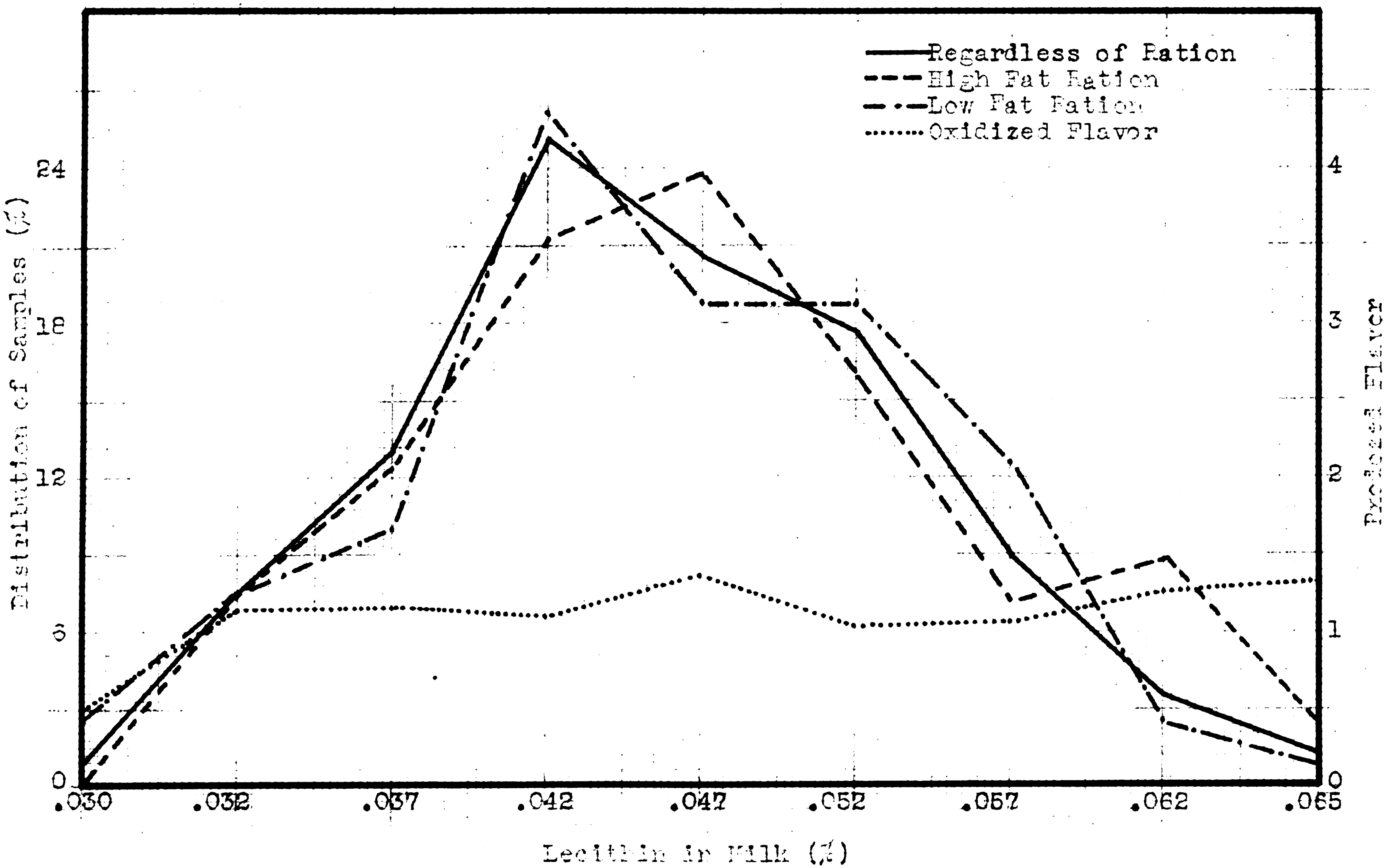
*Key -- 48 hours at 38°F:
- No oxidation
? Questionable
+ Distinctly oxidized flavor
++ Badly oxidized flavor
+++ Very badly oxidized flavor

<u>The percentage distribution of lecithin in milk as affected by the ration.</u> Further analysis of the relationship between the ration and the lecithin content of the milk is given in Tables 11 and 12 and in Graphs 1 and 2.

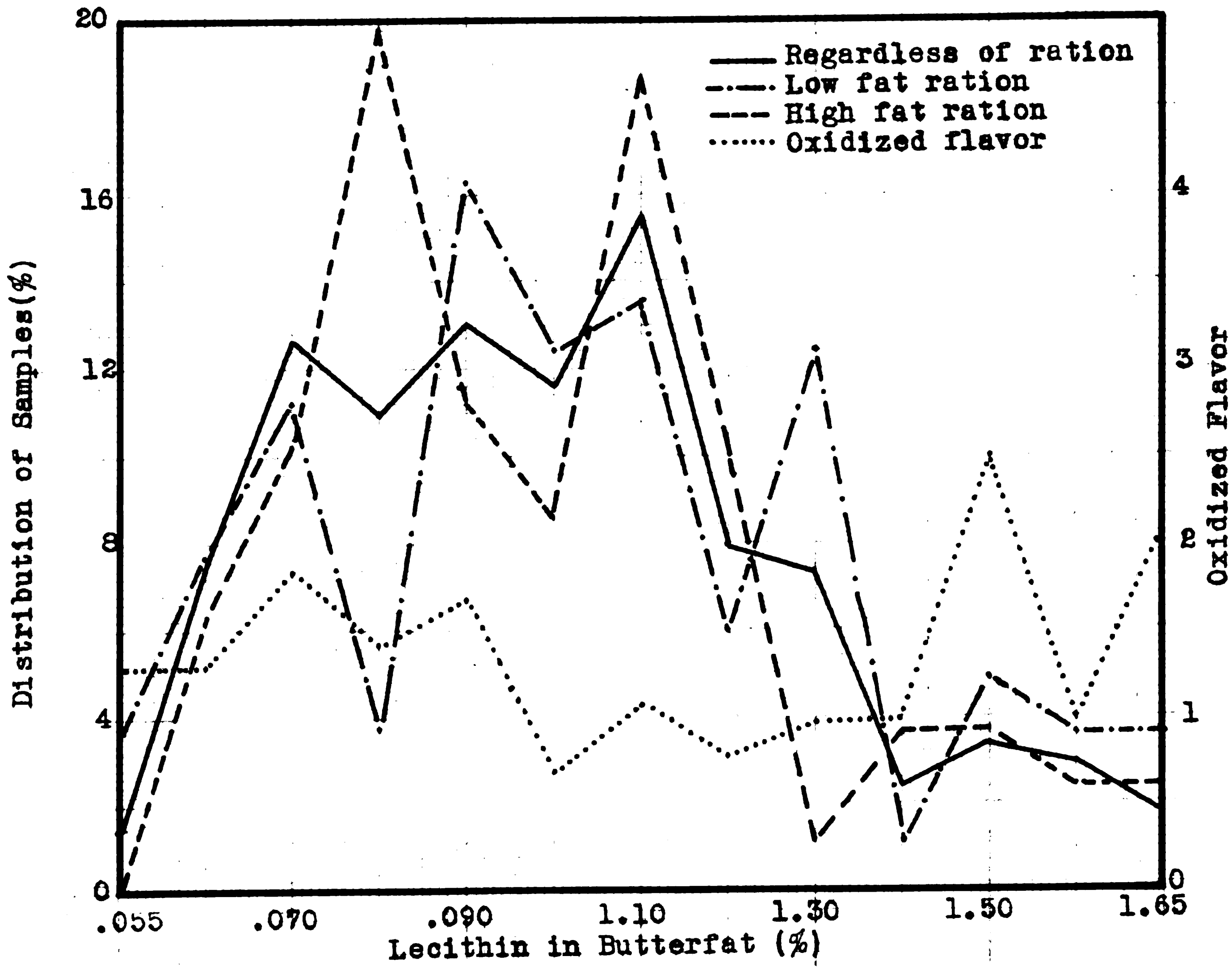
<u>Distribution of lecithin in the milk.</u> The number of samples which fell in the various class intervals over the range of the per cent lecithin in the milk, also the percentage distribution which this number represents of the total number of samples in the group is shown in Table 11. The percentage distribution of lecithin in the milk produced from the various rations as well as the oxidized flavor is presented in Graph 1. The graph shows that the samples analyzed for lecithin fell in a normal distribution over the range established for the per cent lecithin in milk. This is true regardless of whether the cows were on a high or a low fat ration, or whether all of the different samples were considered regardless of the ration. Because of the high percentage values caused by the small total number of samples taken when the cows were on corn, on regular dairy ration, and on pasture these values were not graphed separately. However, as can be seen in Table 11 the distribution is identical with that indicated when all samples were taken together. These values were included with the others in establishing the distribution curve for the lecithin regardless of ration, making a total of 199 samples studied.

Table 11. Distribution of Lecithin in Milk as Affected by the Ration of the Cow.

Mid point lecithin class interval (%)	Distribution of Samples according to Lecithin in Milk when Cow Received:											
	High fat ration		Low fat ration		Corn and alfalfa		Bal. dairy ration		Pasture		Regardless of ration	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Below 0.030	-	-	2	2.50	-	-	-	-	-	-	2	1.0
0.032	6	7.50	6	7.50	1	6.66	2	16.66	-	-	15	7.5
0.037	10	12.50	8	10.00	3	20.00	2	16.66	3	25.00	26	13.1
0.042	17	21.25	21	26.25	5	33.33	3	25.00	4	33.33	50	25.1
0.047	19	23.75	15	18.75	3	20.00	2	16.66	2	16.66	41	20.6
0.052	13	16.25	15	18.75	2	13.33	2	16.66	3	25.00	35	17.6
0.057	6	7.50	10	12.50	1	6.66	1	8.33	-	-	18	9.0
0.062	7	8.75	2	2.50	-	-	-	-	-	-	7	3.5
Above 0.065	2	2.50	1	1.25	-	-	-	-	-	-	3	1.5
Total	80		80		15		12		12		199	



GRAPH 1. EFFECT OF DIFFERENT RATIONS ON THE DISTRIBUTION OF LECITHIN AND ITS RELATIONSHIP TO THE OXIDIZED FLAVOR IN MILK



Graph 2. EFFECT OF DIFFERENT RATIONS UPON THE DISTRIBUTION OF LECITHIN IN FAT AND ITS RELATIONSHIP TO THE OXIDIZED FLAVOR

Distribution of lecithin in the fat. The same type of analysis as presented in Table 11 and Graph 1 is given in Table 12 and Graph 2 except they are concerned with the distribution of samples based upon the per cent lecithin in the fat rather than upon the per cent lecithin in the milk.

The distribution of samples based on the per cent lecithin in the fat is not a normal distribution as is shown by Graph 2. The ration seemed to have little effect on the number or percentage of samples which fell in any interval of the per cent lecithin in fat. Again as in the case of Graph 1 only the high and low fat ration and the aggregate of all samples were graphed. A comparison of the oxidized flavor line and the curve established for the distribution of samples regardless of ration shows that there might be a slight relationship between the two. That is to say, there seems to be a slight relationship shown between the per cent lecithin in the fat of the milk and the intensity of the oxidized flavor which develops in that milk, although evidence herein presented is not sufficient to establish a relationship of any importance.

The relationship between lecithin and oxidized flavor. The lecithin content of the various samples of milk studied throughout the entire experiment are given in Tables 1 - 10 inclusive. There values are expressed both as per cent of lecithin in milk and as per cent of lecithin in fat. As has already been stated the arithmetic mean of these lecithin values was found to be 0.046 per cent for the former and 0.97 per cent for the latter. The oxidized flavor is also given in these tables. A study of the tables shows that oxidized flavor sometimes developed regardless of whether the milk or fat had a high or low lecithin content. A high or a low lecithin content did not necessarily indicate that a sample would become oxidized.

The distribution of all samples according to the number of samples which fell in the various class intervals of lecithin content in the milk according to the intensity of the oxidized flavor which developed in each is given in Table 13.

The distribution according to the per cent lecithin in fat is given in Table 14.

These tables show that oxidized flavor developed over the entire range of the lecithin percentages in all degrees of intensity. The weighted flavor averages given in Table 13 are graphed as the oxidized flavor line in Graph 1 and those in Table 14 are plotted in Graph 2.

These oxidized flavor lines when compared with the curves presented for the distribution of lecithin show very little relationship between the oxidized flavor and the lecithin content of the samples studied.

Table 13. The Distribution of Oxidized Flavor Samples throughout the Various Feeding Trials According to the per cent Lecithin in the Milk.

Mid point lecithin class interval (%)	No. of samples falling in each flavor classification: -	?	+	++	+++	Weighted flavor values*
Below 0.030	1	1	0	0	0	.50
0.032	6	4	2	0	2	1.14
0.037	10	7	4	3	2	1.15
0.042	21	10	8	8	4	1.09
0.047	14	9	7	6	5	1.36
0.052	17	6	7	4	1	1.03
0.057	6	6	3	4	0	1.05
0.062	2	4	1	0	1	1.25
Above 0.065	0	2	1	0	0	1.35

*Key:
- = 0
? = 1
+ = 2
++ = 3
+++ = 4

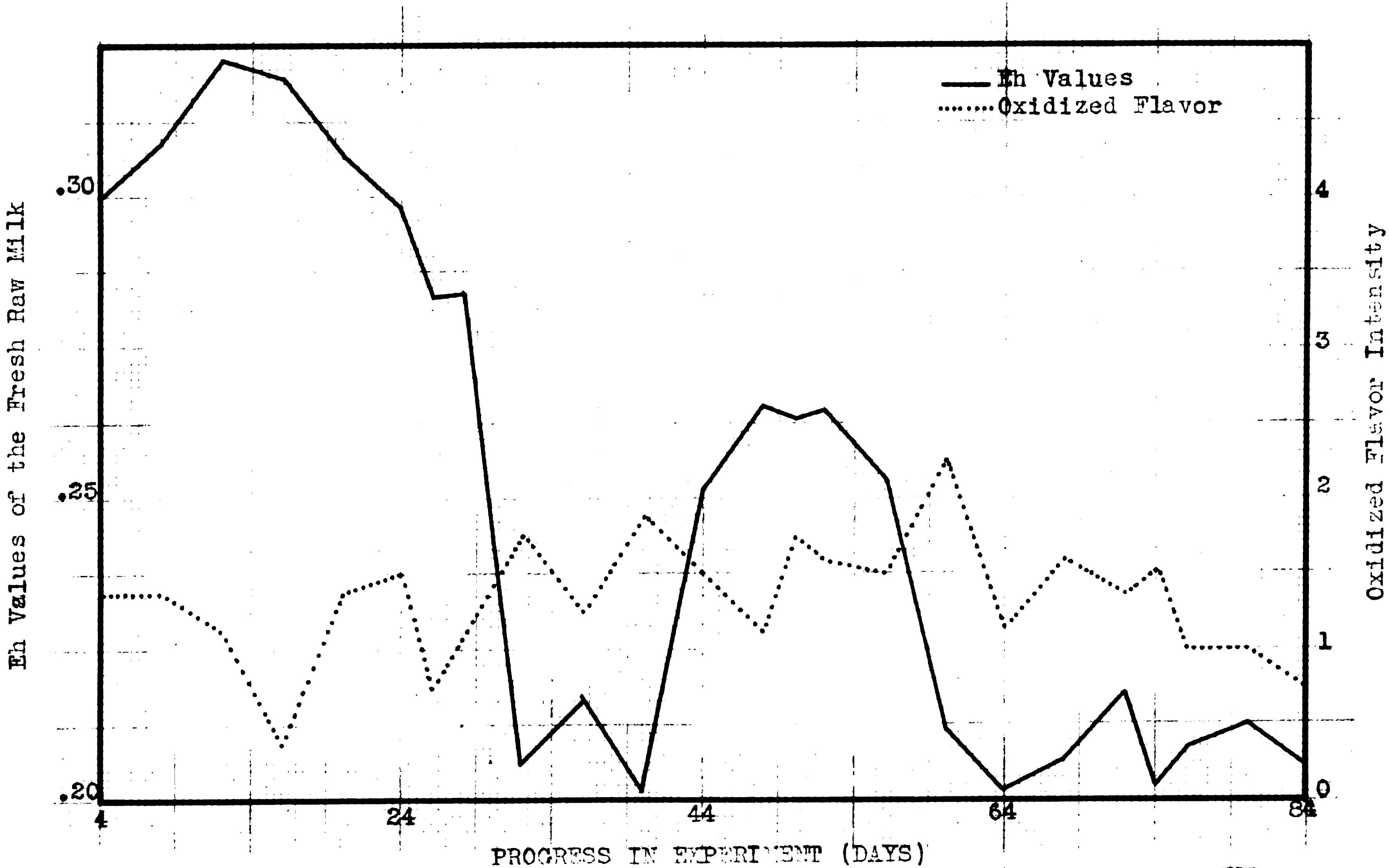
Table 14. The Distribution of Oxidized Flavor Samples Throughout the Various Feeding Trials According to the Per cent Lecithin in the Fat.

Mid point lecithin class interval (%)	No. of samples falling in each flavor classification:					
	-	?	+	++	+++	Weighted flavor values*
Below 0.55	0	2	1	0	0	1.33
0.60	4	5	3	3	0	1.33
0.70	8	9	4	3	4	1.85
0.80	7	6	3	4	2	1.45
0.90	10	3	7	5	0	1.68
1.00	11	9	2	1	0	.72
1.10	14	8	3	4	2	1.09
1.20	9	2	4	1	0	.81
1.30	9	2	1	1	3	1.00
1.40	1	3	1	0	0	1.00
1.50	0	1	2	2	1	2.50
1.60	3	2	0	0	1	1.00
Above 1.65	1	0	1	2	0	2.00

*Key:
- = 0
? = 1
+ = 2
++ = 3
+++ = 4

<u>The relationship of oxidation-reduction potentials to oxidized flavor</u>. The oxidation-reduction potentials, or Eh values, for each of the individual samples of fresh raw milk was determined in duplicate by the use of a bright foil platinum electrode and a saturated calomel cell. The EMF readings were made on a Leads and Northrop portable polentiometer and converted to Eh values by resolving to a normal hydrogen electrode basis. The average of these values are recorded in Tables 1 to 10 inclusive, as previously given.

The trend of the Eh values throughout the different feeding periods with the same cows is shown in Graph 3. The values graphed were obtained by averaging the Eh values for all of the samples on a particular day. The graph shows considerable fluctuation in the Eh values of the samples examined. The general tendency was toward a constant reduction in oxidation-reduction potential throughout the entire experiment. The same reduction was observed in the samples when the special group of cows were on pasture, as recorded in Table 10. Presented in Graph 3, also, is the oxidized flavor line. In this manner Graph 3 shows both the average Eh value and the average oxidized flavor value for the same sample of milk every day that samples were taken. Inspection of this graph indicates that little to no relationship between the oxidation-reduction potential of the individual milk samples and the development of oxidized flavor existed in this experiment.



GRAPH 3. RELATIONSHIP BETWEEN THE OXIDATION-REDUCTION POTENTIALS AND THE DEVELOPMENT OF OXIDIZED FLAVOR

<u>Effect of feed on oxidized flavor development</u>. The 48-hour oxidized flavor scores on the various samples of milk taken from the cows receiving the different rations throughout the experiment are given in Tables 1 to 10 inclusive. From these tables it can be seen that little relationship existed between the rations used in this experiment and the oxidized flavor which developed in the milk produced, except in individual cases. Further analysis of the relation between these rations and the oxidized flavor may be found in Tables 15 to 20 inclusive.

The rate of change in oxidized flavor after the first reversal in ration for each of the cows in the high and in the low fat double reversal experiment is shown in Table 15. Cow 7 is shown to have given milk which developed a very badly oxidized flavor in the previous period but at no time during the low fat feeding period which followed was the milk scored more than questionable after pasteurization and holding. The milk from Cow 66 following the change in the ration showed very little change in the flavor which developed. The same was true of Cows 242 and 246. The milk from Cows 99, 101, and 218 showed an increase in the tendence to develop oxidized flavor when they were changed from the low fat ration to the high fat ration. The milk from Cow 171 showed an increase in intensity of oxidized flavor development on the reverse change in ration.

Table 15. The Rate of Change in Oxidized Flavor After the First Reversal in Ration

Cow number	Nature of* ration change	Oxidized flavor** in sample taken the following number of days after reversal of ration							
		0	2	4	8	12	16	20	24
7	High to Low Fat	+++	-	?	?	?	-	?	-
66	" " " "	?	-	-	+	-	-	-	-
171	" " " "	-	-	-	?	-	++	++	-
246	" " " "	+	-	-	-	-	+	-	-
99	Low to High Fat	+	-	-	+	++	?	+	+++
101	" " " "	?	-	-	+++	++	-	-	+
218	" " " "	?	-	++	++	+	+++	++	+
242	" " " "	+	-	-	?	+	-	+	?

* Double reversal system used.
** Key -- (48 hours at 38°F.):
- No oxidized flavor
? Questionable
\+ Distinctly oxidized yet not objectionable
++ Badly oxidized
+++ Very badly oxidized

The rate of change in oxidized flavor after the second reversal in ration is shown in Table 16. The table shows that when cow 7 was changed from a low to a high fat ration a change in intensity of the oxidized flavor which developed in the milk was noted on the reversal day; and that the intensity became very bad before the end of the feeding period. The milk from cows 66, 171, 218,and 246 is shown to have changed very little in susceptibility to oxidized flavor development. The milk from cow 99 is shown to have decreased slightly in the intensity of oxidized flavor developed when she was changed from the high to the low fat ration. On the fourth day the flavor development had cleared up only to return slightly and then disappear before the end of the feeding period. The milk from cow 101 showed little change in flavor development after the change from the high to the low fat ration. It was about this time that the regular microscopic examination of the milk from the various animals in the herd disclosed the development of mastitis in cow 101 although her physical condition and observation of the milk did not disclose the infection. The milk from cow 242 showed an increase in the intensity of oxidized flavor developed when her ration was changed to the low fat level.

The rate of change in oxidized flavor developed in the milk from individual cows in the final low fat group was noted when this group was placed upon a ration in which corn

Table 16. The Rate of Change in Oxidized Flavor After the First Reversal in Ration

Cow number	Nature of* ration change	Oxidized flavor** in sample taken the following number of days after reversal of ration							
		0	2	4	8	12	16	20	24
7	Low to High Fat	-	?	++	++	+	++	+++	+++
66	" " " "	-	-	?	+	+	-	-	-
171	" " " "	-	-	-	-	-	-	-	-
246	" " " "	-	-	+	-	++	?	?	-
99	High to Low Fat	+++	++	-	-	?	?	-	-
101	" " " "	+	++	+	++	++	?	+	-
218	" " " "	+	+++	++	+	+++	++	+++	+++
242	" " " "	?	+	+	?	++	-	++	+

* Double reversal system used.
** Key -- (48 hours at 38°F.):
- No oxidized flavor
? Questionable
+ Distinctly oxidized yet not objectionable
++ Badly oxidized
+++ Very badly oxidized

Table 17. The Rate of Change in Oxidized Flavor When the Cows were changed from a Low Fat Ration to a Ration of which Corn was the only Grain.

Cow number	Oxidized flavor* (at 48 hrs.) in sample taken: the following number of days after reversal.				
	0	2	4	8	12
99	-	?	?	+	-
101	-	?	(lost)	?	?
218	+++	+	?	?	+
242	+	+	?	-	-

Key:
- \- No oxidized flavor
- ? Questionable
- \+ Distinctly oxidized flavor
- ++ Badly oxidized flavor
- +++ Very badly oxidized flavor

alone was the sole source of grain. The data obtained are recorded in Table 17. This table shows that in the case of cows 99 and 101 previously giving milk free of the susceptibility to oxidized flavor, there was a tendency for the milk to develop oxidized flavor on the corn ration. The milk of cows 218 and 242, however, showed a tendency toward a decrease in the intensity of oxidized flavor developed, although the milk of cow 218 did not entirely lose the susceptibility to the flavor development.

A summary of the experiment in regard to oxidized flavor and rations is given in Table 18. The flavor scores given are averages of the different samples taken during the specified period and were obtained by assigning each flavor score a relative numerical value and averaging these values. The table shows that the milk from cows 7, 66, 99 and 246 had a tendency to develop a more pronounced oxidized flavor on the high fat ration than on the low fat ration. The milk of cows 101, 218 and 242 developed a more intense oxidized flavor on the high fat ration after having been on the low fat ration for the first period but retained the same intensity of oxidized flavor development after the group had been returned to a low fat ration in the final feeding period of the double reversal trials. The milk from cow 171 (highly mastitic) was shown to be entirely free from oxidized flavor on the high fat ration but to develop a badly oxidized flavor on the low fat ration.

The development of oxidized flavor before and after

Table 18. Effect of High and Low Fat Ration on Oxidized Flavor Development in Milk.

Order of feeding trials	Nature of ration*	Oxidized flavor developed after 48 hours at 38°F.** (Average 6 samples taken every 4th day during 24 day periods)*** Cow 7	Cow 66	Cow 99	Cow 101	Cow 171	Cow 218	Cow 242	Cow 246
1	High Fat	+++	?			-			++
2	Low Fat	?	-	+	+	++	+	?	-
3	High Fat	+++	?	++	++	-	+++	+	+
4	Low Fat			?	++		+++	+	
5	Corn Only			+	+		+	?	

* Double reversal system used.

** Key:

- \- No oxidized flavor
- ? Questionable
- \+ Distinctly oxidized yet not objectionable
- ++ Badly oxidized (draw complaint)
- +++ Very badly oxidized

*** Only last two samples taken in first period were treated with $CuCO_4$ hence only these two are used in this table.

Table 19. Oxidized Flavor Before and After the Addition of Grass to the Ration of Four Representative Cows.

Cow No.	Flavor development (at 48 hours) Sample taken before grass						Flavor development (at 48 hours) Sample taken after grass					
	8 days		4 days*		1 day							
	lbs	Flavor	lbs.	Flavor	lbs	Flavor	lbs	Flavor	lbs.	Flavor	lbs	Flavor
25	13.1	?	14.3	?	14.0	+	14.2	-	18.8	?	18.0	-
94	8.7	?	8.7	?	9.0	-	10.0	+	11.0	+	8.1	-
134	13.6	-	11.0	-	11.3	-	12.6	-	15.3	-	13.3	-
193	23.4	-	12.2	-	18.7	-	16.0	-	17.0	-	14.2	-

* Key:
- No oxidation
? Questionable
\+ Distinctly oxidized flavor
++ Badly oxidized flavor
+++ Very badly oxidized flavor

the special group of cows were placed on pasture in the spring is shown in Table 19. The table shows the number of pounds of milk produced at the noon milking from which the samples were taken and the oxidized flavor score after forty-eight hours. The milk from both the Guernsey and the Jersey was susceptible to the development of oxidized flavor and did not lose the susceptibility until after the cows were on pasture ten days. The other two cows gave milk which did not develop oxidized flavor at all.

The distribution of samples which developed the various intensities of oxidized flavor according to the ration which the cows received is presented in Table 20. The weighted numerical average of the oxidized flavor development for Group 1 on the high fat ration was 1.1 which means milk of questionable oxidized flavor. The average for Group II on high fat ration was 2.2 which is slightly above one plus in oxidized flavor intensity. The milk from Group I averaged 0.32 on oxidized flavor intensity while the group was on the low fat ration. Group II showed an average of 2.15 oxidized flavor development in the milk when they were on the low fat ration.

Susceptibility of the milk from each group of cows to the development of oxidized flavor. The effect which each group of cows on the double reversal high and low fat ration experiment had upon the development of the oxidized flavor in the milk is shown in Table 21.

Table 20. Effect of Ration on Intensity of Oxidized Flavor Development in Individual Milk Samples.

Identification			Number of samples which developed the following flavor intensity.					Weighted flavor*
Period	Group	Ration	-	?	+	++	+++	average
First	I	High fat	8	12	2	1	1	1.00
"	II	Low fat	5	7	8	3	1	1.50
Second	II	High fat	3	6	5	10	4	2.20
"	I	Low fat	21	5	2			0.32
Third	I	High fat	13	4	5	4	2	1.20
"	II	Low fat	6	4	5	8	5	2.08
Fourth	II	Corn	3	8	4	-	-	1.06
-	Special	Balanced	8	3	1	-	-	0.42
-	Special	Pasture added	9	1	2	-	-	0.42

*Key:
- = 0
? = 1
+ = 2
++ = 3
+++ = 4

Table 21. Comparison of the Milk from the Cows in Each Group as to the Intensity of Oxidized Flavor Developed.

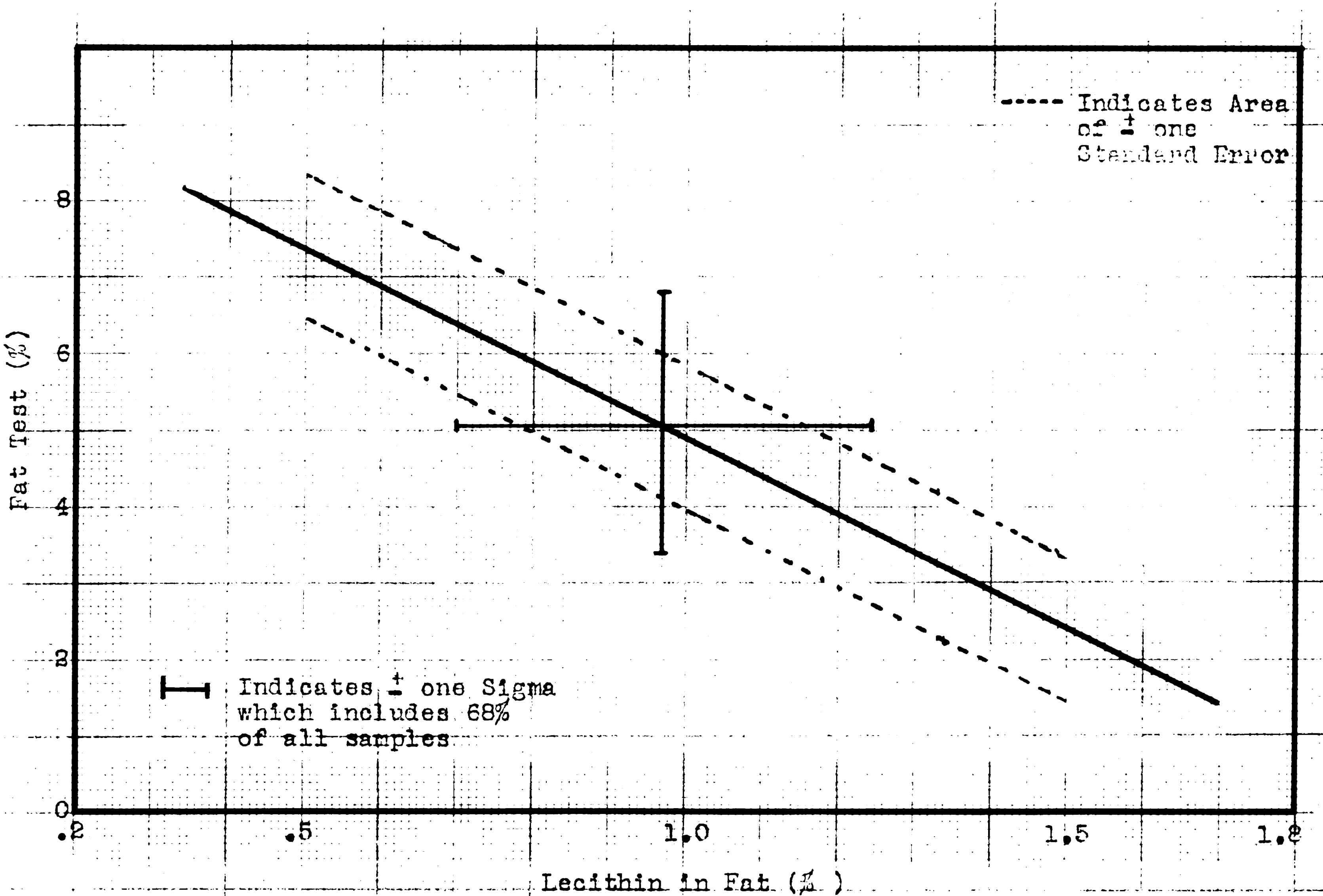
Nature of ration	Number of samples which developed the following intensity of oxidized flavor: -	?	+	++	+++	Weighted flavor* average
			Group I			
High fat	8	12	2	1	1	1.00
Low fat	21	5	2	-	-	0.32
High fat	13	4	5	4	2	1.20
			Group II			
Low fat	5	7	8	3	1	1.50
High fat	3	6	5	10	4	2.20
Low fat	6	4	5	8	5	2.08

* Key:
- = 0
? = 1
+ = 2
++ = 3
+++ = 4

The first group composed of cows 7, 66, 171, and 246 is shown to have produced milk which developed a much lower oxidized flavor intensity than did Group II composed of cows 99, 101, 218, and 242.

This study shows that the individual animal played an important part in the intensity of the oxidized flavor which developed.

<u>Analysis of data presented by means of the correlation table</u>. A study of Tables 1 to 10 inclusive suggested a relationship between the fat test of the milk and the per cent lecithin in the fat. Consequently, a correlation table was set up and the number of samples falling within the various class intervals tallied. The arithmetic mean of the per cent fat was found to be 5.049 per cent with a standard deviation of 1.661 per cent while the arithmetic mean of the per cent lecithin in fat was 0.9705 per cent with a standard deviation of 0.2697 per cent. The coefficient of correlation, which is the measure of relationship between two groups of figures, was figured to be -0.8248 and the standard error of estimate, which is the average of the deviations about the line of regression, was figured to be 0.9393. From this data the line of regression was determined and later corrected to original values. The corrected regression line followed the equation $y=-5.08x+9.979$. The line of regression showing the standard error is given in Graph 4 as are the two



GRAPH 4. CORRELATION BETWEEN FAT TEST AND PER CENT LECITHIN IN FAT
COEFFICIENT OF CORRELATION —.8245, STANDARD ERROR .94

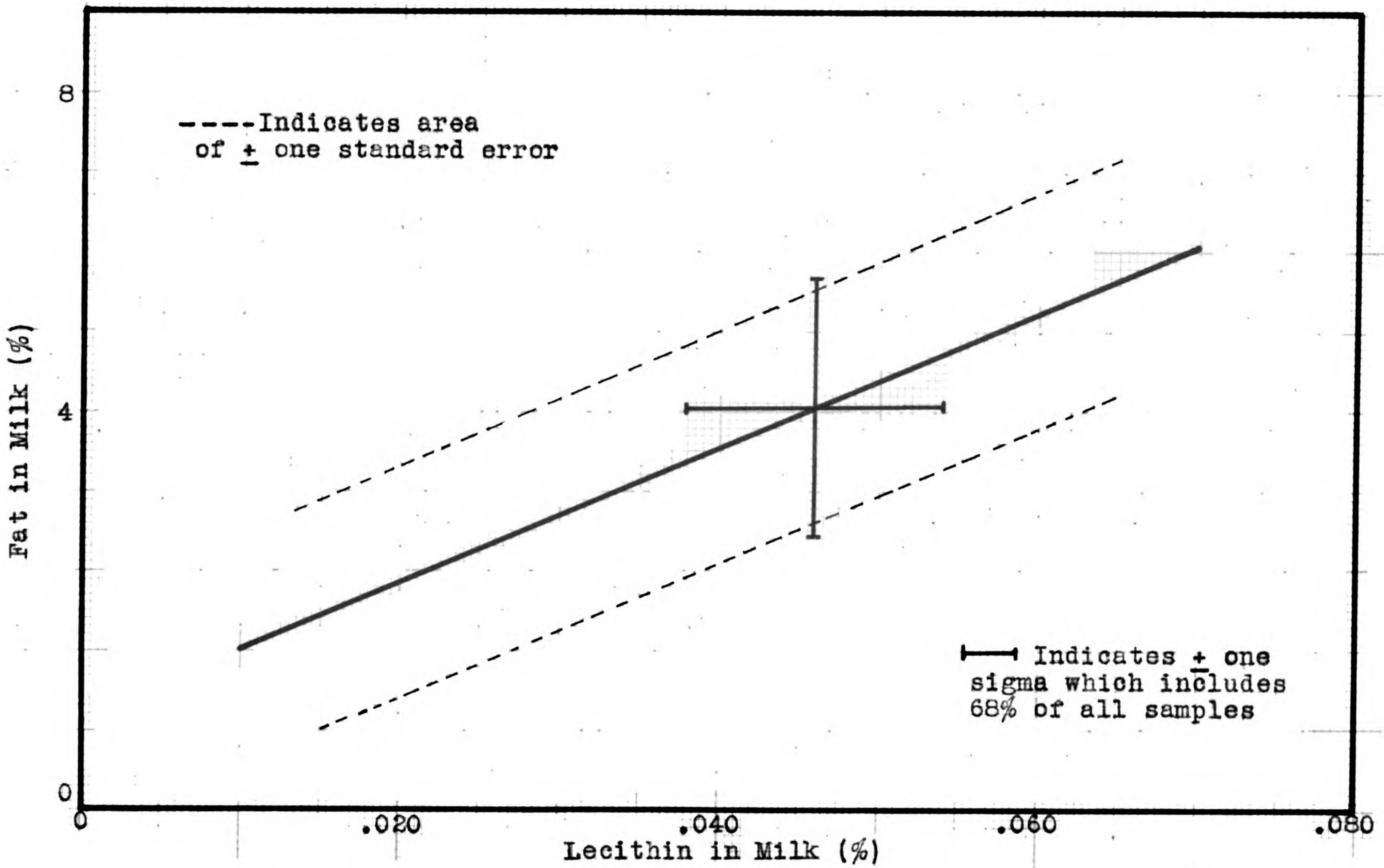
arithmetic means of the components with their standard deviation indicated. This graph shows that as the per cent fat in the milk increased there was a definite decrease in the per cent lecithin in the fat. The high coefficient of correlation in this study makes the line of regression quite significant. This study might seem to indicate that there was only a definite amount of lecithin produced in the milk regardless of the amount of fat.

Another correlation table was made for the per cent lecithin in milk against the fat test. In this correlation the arithmetic mean of the fat test was 5.002 per cent with a standard deviation of 1.619 per cent. The arithmetic mean of the per cent lecithin in milk was 0.0459 per cent with a standard deviation of 0.008 per cent. The coefficient or correlation, r, in this study figures 0.411. The standard error of estimate was 1.4764. The equation for the line of regression was determined to be $y=83.217x+1.174$ when corrected to the original values.

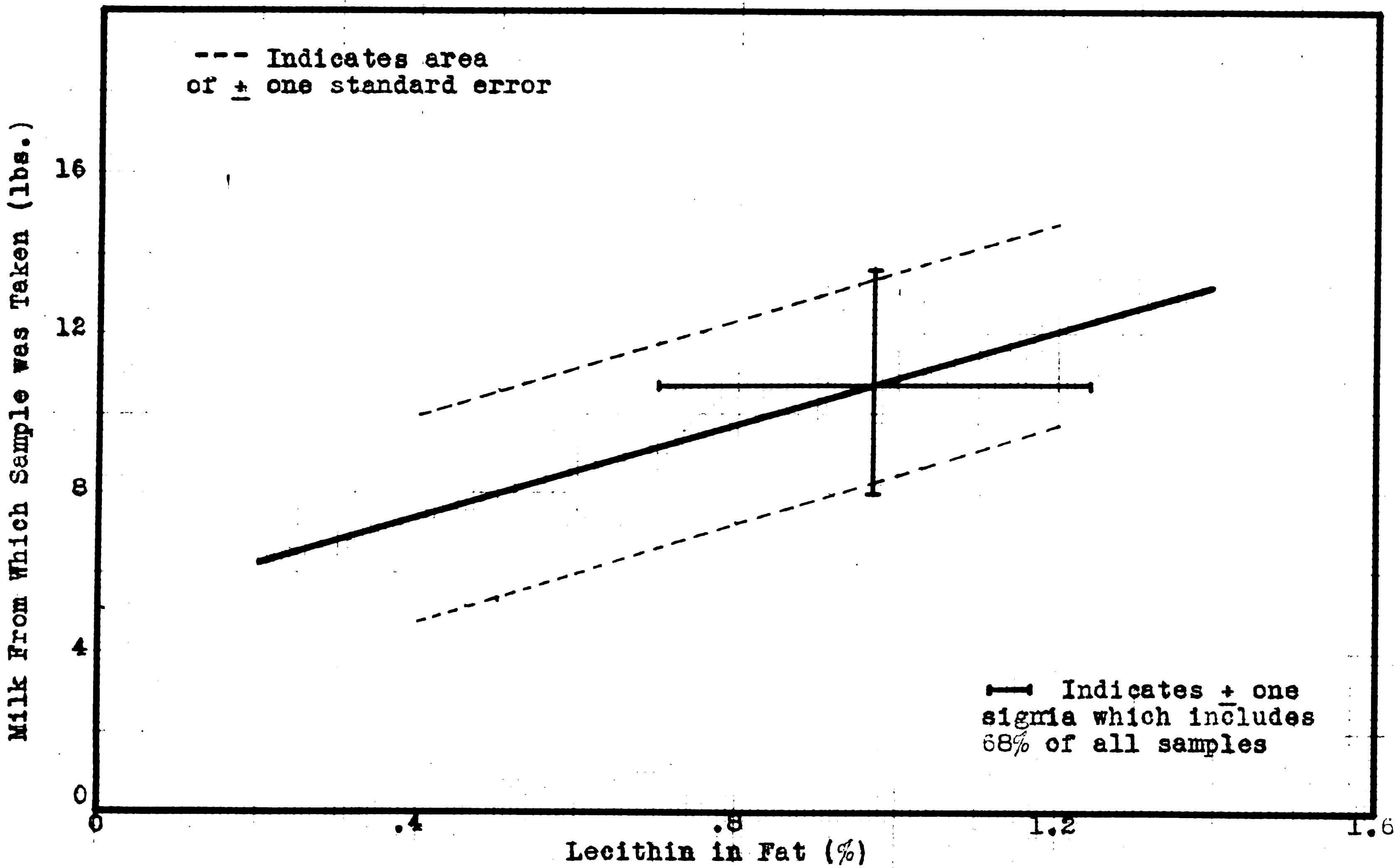
The line of regression of the per cent fat - per cent lecithin in milk correlation with the area of the standard error, the arithmetic means of the two conponents and their respective areas of standard deviations are shown in Graph 5. The graph shows that with an increase in the fat test there is also an increase in the per cent lecithin in the milk.

A study was made of the relationship between the lecithin content of the fat, the lecithin content of the milk, and the pounds of milk which the cow gave at the milking from which the samples were taken. The correlation between pounds of milk produced and the per cent lecithin in the fat showed that the arithmetic mean of the milk produced was 10.72 pounds with a standard deviation of 2.898 pounds. The arithmetic mean of the per cent lecithin in fat was 0.9705 per cent with a standard deviation of 0.2713 per cent. The coefficient of correlation in this study was 0.4144 which was slightly higher than that of the per cent fat - per cent lecithin in milk correlation. The standard error of estimate was found to be 2.6372. The equation for this line of regression was $y=4.297x+5.151$. These data are presented in graphic form in Graph 6. There seemed to be a tendency for the per cent lecithin in fat to increase as the amount of milk which the cow produced increased.

No significant correlation existed between the pounds of milk produced and the per cent lecithin in the milk.



Graph 5. CORRELATION BETWEEN PER CENT FAT AND PER CENT LECITHIN IN MILK
COEFFICIENT OF CORRELATION .411, STANDARD ERROR OF ESTIMATE 1.476



Graph 6. Correlation Between Pounds of Milk and Per cent Lecithin in Fat.
Coefficient of Correlation .4144, Standard error of Estimate 2.637

DISCUSSION

Effect of high and low fat ration on lecithin content, oxidized flavor and oxidation-reduction potentials of the milk. There seems to be a lack of information in the literature on the subject of the relationship of the lecithin content of milk to the susceptibility of the milk to the development of oxidized flavor. The results herein reported on the effect of the soy bean oil, which was used to increase the fat content of the ration in this experiment on the oxidized flavor, are in contrast to those of Prewitt (28). However, there was some difference in the type of basal ration used. In the high and low fat experiment herein presented the basal ration consisted of good quality alfalfa hay, beet pulp, solvent extracted soy bean flakes, bone meal, and molasses. The fat level was raised by the addition of soy bean oil in proportion to the fat content of the milk from each cow. Neither of the levels of fat in the ration used in this experiment seemed to effect the percentage of lecithin expressed either on the basis of milk or of fat.

The oxidation-reduction potentials fluctuated considerably throughout the high and low feeding trials, but as has already been pointed out the general tendency of the trend was toward a constant decrease. The peak potential reached 0.3296 volts and the decrease was gradual over the seventy-two day period until at the end a potential slightly above 0.20 volts was reached. The level of fat in the ration seemed to have little or no effect upon the susceptibility of the milk produced to the development of the oxidized flavor. The oxidized flavor developed in many instances in milk produced on either ration regardless of lecithin content or oxidation-reduction potential, where it was pasteurized with 2.5 ppm copper and then stored. The fact that oxidized flavor developed regardless of the oxidation-reduction potential of the fresh raw milk is in perfect agreement with the work of Webb and Hileman (46).

Effect of corn as the only grain in the ration upon the lecithin content, oxidized flavor development, and oxidation-reduction potentials of the milk. This type of ration seemed to have no effect upon the lecithin content of the milk which was produced, upon the oxidation-reduction potential, or upon the oxidized flavor development. Lecithin values and oxidation-reduction potentials fluctuated in a similar manner as they had fluctuated during the high and low fat ration feeding trials. The oxidized flavor developed in the milk regardless of lecithin content or oxidation-reduction potential just as it had in the previous low and high fat feeding periods.

Effect of the addition of pasture to the ration upon the lecithin content, oxidized flavor development, and oxidation-reduction potentials of the milk. The lecithin content of the milk produced by the special group of cows on both the regular dry balanced dairy ration and the ration supplemented by pasture was practically the same. The addition of pasture to the ration did not decrease the lecithin content of the milk nor did it cause a pronounced drop in the oxidation-reduction potential of the milk. The experiment was thought to have been extended sufficiently long to establish what effect the pasture would have upon the elimination of the susceptibility of the milk to oxidized flavor development. However, the 10 days of pasture feeding was not sufficiently long to inhibit the development of the oxidized flavor entirely.

The lecithin content, oxidized flavor, and oxidation-reduction potentials of the milk regardless of ration. The average lecithin content of milk as expressed on the basis of per cent lecithin in milk and per cent lecithin in fat was found to be slightly higher than that observed by other investigators although the range of these values corresponds very favorably with their findings. The slightly higher result may be explained on the basis of one or two facts. First, and most likely, is the presence and influence of mastitis upon the lecithin contents herein reported. This theory is based upon the statement of Horrall (21) that mastitic cows gave milk with much higher lecithin content than did normal cows. On the other hand the greater number of samples analysed and the longer period of time covered may have had considerable bearing upon these values. The oxidation-reduction potentials in every instance showed a trend toward a gradual decrease with a high potential of 0.3379 volts and a low potential of 0.1335 volts. The individuality of the cow seemed to have more to do with the development of oxidized flavor in the milk than any other single factor. The latter contention is in agreement with the work of Rasmussen and others.

The fact that oxidized flavor seemed to develop in this experiment regardless of ration when others (8, 6, 28) found that the addition of certain feeds would eliminate the susceptibility to this flavor from the milk may best be explained on the basis of difference in the rations used.

Correlations. The correlations presented offer a special treatment of the data in an effort to establish a definite mathematical relationship between the lecithin content and the fat content, the oxidation-reduction potentials and the development of oxidized flavor. The correlation between the per cent fat and the per cent lecithin in the fat is quite significant showing a definite inverse relationship. In fact, the significance is of such magnitude that within certain small deviations the per cent lecithin in the fat might be predicted from the fat test of the milk by the use of the formula for the line of regression.

The other correlations given are somewhat less significant.

SUMMARY

1. One hundred and ninety-nine samples of milk from individual cows were analyzed in duplicate for lecithin content, oxidation-reduction potentials, and oxidized flavor development.

2. The lecithin content expressed as per cent in milk and as per cent in fat was shown to be subject to considerable fluctuation over any extended period of time. Sixty-eight per cent of the samples were found to contain 0.0448 $\pm$ 0.008 per cent lecithin in the milk, or as expressed in per cent lecithin in fat, 0.9705 $\pm$ 0.2697 per cent lecithin in the fat.

3. The oxidized flavor development in the milk was also shown to fluctuate throughout the entire experiment.

4. Neither the high nor the low fat ration used in this experiment was shown to have any noticeable effect upon the lecithin content of the milk or upon the oxidized flavor which developed in the milk.

5. Corn, when used as the sole grain in the ration, caused no definite change in the lecithin content and had no detectable influence upon the susceptibility of the milk to the oxidized flavor.

6. The addition of pasture to the ration of four representative cows previously receiving a dry balanced ration was shown to have no effect upon the per cent of

lecithin produced. Two of the four animals produced milk which did not develop oxidized flavor either on the dry ration or after pasture was added. The other two animals were shown to give milk which developed oxidized flavor on both the dry and on the pasture supplemented rations.

7. Little relationship was found between the rations of the experiment and the oxidized flavor which developed in the milk.

8. Oxidation-reduction potentials of fresh raw milk from individual cows showed no relationship with the lecithin content or with the oxidized flavor which developed in the milk after pasteurization.

9. Individuality of the cow was shown to be a decided factor in the lecithin content of the milk and the oxidized flavor which developed.

10. A significant correlation was shown between the per cent lecithin in fat and the fat test of the milk. The coefficient of correlation -.8248 with a standard error of estimate of .9393 showed that within a very narrow range of deviation the per cent lecithin in fat was inversely proportional to the per cent of fat.

11. The correlation between the per cent lecithin in milk and the fat test of the milk showed a coefficient of .4110 and a standard error of 1.4764. This shows that the per cent lecithin in milk was directly proportional to the fat test although not so significant as the previous correlation.

12. The correlation between the pounds of milk produced at the noon milking at which time all samples were taken and the per cent lecithin in the fat showed a coefficient of correlation of .4114 and a standard error of estimate of 2.6372.

13. No correlation was found to exist between the pounds of milk and the per cent lecithin in milk.

14. There was no relationship between the lecithin content of the milk and the oxidized flavor developed. Oxidized flavor developed in milk of either high or low lecithin content.

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