Influence of soybeans on the flavor of milk, cream and butter and on the body and texture of butter

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UMI
INFLUENCE OF SOYBEANS ON THE FLAVOR OF MILK, CREAM AND BUTTER AND ON THE BODY AND TEXTURE OF BUTTER

by

Jennings Bryan Frye, Jr.

A Thesis Submitted to the Graduate Faculty for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Dairy Husbandry

Approved:

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I. INTRODUCTION

It is most gratifying to note the unique role that dairy products have occupied through the ages in the diet of man, especially in the diets of the more highly developed and civilized peoples, such as the Greeks and Romans. Since the Christian era, and with the advancement of civilization in all parts of the world, there has been an upward trend in the number and quality of dairy cattle. In the United States, at the present time, there are nearly 87 million dairy cows producing over 118 billion pounds of milk per year. In spite of this, the per capita consumption of this "most nearly perfect food" is far below the standard set forth by nutritionists.

Increased consumption of dairy products depends upon several factors, of which the most pertinent to this experiment have been grouped under one heading, that of quality. Since milk is the basis of all dairy products, it is fundamental that considerable attention be given to its method of production and processing in order to produce high quality products. It is encouraging that the more progressive milk plant managers and dairymen are trying to cope with the
numerous factors relevant to quality so that milk products of the highest type may be produced.

Quality milk, to the average consumer in the past, has meant a deep cream line and a desirable flavor. However, with the advent of homogenization, a better informed consumer, and the use of paper containers, there is a tendency to subordinate the significance of a cream line and to give more attention to the flavor. It is, therefore, of primary importance that milk, as well as other dairy products, possess a clean, desirable flavor.

Quality, to the average consumer in the past, has had a variety of meanings, depending largely upon the section of the country in question and the grade of product usually available for consumption. However, with the advancement of science and our educational facilities, such as the extension services, breed organizations, and short-courses, for dissemination of information to the public, consumers of dairy products quite generally are demanding a product which complies with the highest standards of nutrition, sanitation, and flavor. Comparatively speaking, the search for better feeding, dairy farm management, and processing practices has scarcely begun in this vastly important and relatively new field - "Quality."
The recent and rapid increase in the production of soybeans and their greater use as a protein supplement for dairy cows has created a strong and insistent demand among dairymen and creamery operators for more information regarding the effect that soybean feeds have on the flavor and quality of dairy products.

In the past few years such questions as the following have arisen: How many pounds of soybeans or soybean products may be fed to a cow daily without producing a deleterious effect on her milk product? If there is a deleterious effect, how soon does it become noticeable in the product after feeding the beans? Do the amounts normally fed by dairymen produce an off-flavor in milk and cause the butter to have a poor body and texture? By a certain proportioning of soybeans and cottonseed meal or soybeans and linseed meal in the ration, is it possible for dairymen to obtain a more desirable butterfat, as to body and texture, during the summer and winter months?

At the present time, soybeans are one of the most readily available high protein concentrates on Iowa farms, as well as in a large part of the United States. Thus, it is not surprising that considerable attention has been directed to the economic feasibility of using soybeans and soybean products in the dairy ration.
This experiment has been designed to answer some of the questions relative to the effect of soybeans on the flavor and quality of dairy products. The objectives are:

1. To determine the time necessary for cracked soybeans to produce a maximum change in the flavor and quality of milk and butterfat.

2. To study the variations in certain butterfat constants throughout a cow's lactation period.

3. To determine the feasibility of using the iodine number as a measure in ascertaining when a feed with a high oil content has exerted its maximum effect.
II. REVIEW OF LITERATURE

A. Undesirable Flavors Occurring in Milk and Cream and Some of Their Causes

Milk as it normally comes from the cow, usually possesses a mild, pleasant, appealing flavor. However, other flavors, which make it less palatable, are often present in the milk, or develop soon after it is obtained. Some interesting work relative to the "taste" of milk has been reported by Roadhouse and Koestler (132). The taste of milk, as referred to in their report, is the sensation perceived when milk is taken into the mouth. The term "flavor" was used to designate a combination of the sensations of taste, perceived in the mouth, with those of smell, produced through the medium of the inner nasal passages. They state that the foundation taste of milk resulting from its natural composition is supplemented by a secondary one. The chloride-lactose relation was found to be one of the most important bases of milk flavor. It was demonstrated by dialysis that fat and protein substances, as well as certain difficulty dialyzable salt components play only a subordinate role in the primary taste of milk. The dialysis of milk containing a pronounced feed flavor revealed that it was largely in the residue and appeared to be either not dialyzable or in some way combined with milk.
fat or with other non-dialyzable materials.

According to Gamble and Kelly (55) the following factors are usually responsible for off-flavors: (1) The internal or physical condition of the cow; (2) absorption of flavors and odors from highly flavored feeds within the body of the cow; (3) absorption of flavors and odors into the milk after production; and (4) bacterial development within the milk.

Because feed and oxidized flavors are those most commonly encountered in milk and butterfat this discussion will involve, primarily, significant aspects relative to their occurrence and correction.

1. Feed Flavor

That the feed consumed by the cow is often the cause of undesirable flavors in milk, has long been recognized. As early as 1889, William Harley (74) described a method for "preventing milk from tasting of turnips." In a recent report, Weaver, et al. (163) emphasize that undesirable feed flavors are the most prevalent of the flavor defects encountered in milk.

The systematic study of Gamble and Kelly (55) of the effect of silage on the flavor and odor of milk stimulated interest in flavor studies. They concluded that the flavor and odor of silage are largely imparted to the milk through the body of the cow, for shortly after as little as ten
pounds were fed, the milk developed a faint feed flavor and odor. They maintained that not over fifteen to twenty-five pounds of corn silage or fifteen pounds of legume silage can be fed twice daily after milking without imparting a discernible flavor and odor to the milk. Feed flavors are not always objectionable. In fact, these authors suggested that if moderate quantities of corn silage are fed after milking and that if the milk is promptly aerated, the flavor may in some cases actually be improved. Since Marshall (103) found that aeration alone improves the flavor of milk, it may be that the objectionable substances imparted to milk by silage are volatile and can be readily removed. This theory was partially supported by Gamble and Kelly.

Another very interesting point brought out by Gamble and Kelly (55) was that condensed milk made from silage-tainted milk has a less perceptible silage flavor and odor than that of the original milk.

Legume silage seems to react differently from corn silage for Woll and Humphrey (169) assert that satisfactory dairy products cannot be made when cows are fed soybean silage. However, Farrington (55) points out that with care, silage (any kind) can be fed to dairy cows without tainting the milk, but unless precautions are taken, the product may be so tainted that many customers will refuse to use it. Woodward and McNulty (170) advocate considerable care to avoid tainting.
the milk when feeding clover silage.

Roadhouse and Henderson (133) detected an alfalfa flavor in milk within 20 minutes after drenching cows with flavor-producing liquids. The most pronounced feed flavor was detected within 45 to 60 minutes after the drenching.

Babcock (9) found that the feeding of green alfalfa three hours before milking decreased the intensity of the abnormal flavor and that feeding five hours before milking practically eliminated it.

Babcock (15), Roadhouse and Henderson (135) ascertained that when rations of green alfalfa, alfalfa hay, clover hay, or corn silage are fed one to two hours before milking, strong, undesirable feed flavors and odors result in the milk. When eight to nine pounds of tame oat hay were fed two hours before milking, only a slight off-flavor appeared in the milk.

In general, it is recommended (28, 77, 98, 135, 163) that cows be removed from alfalfa pasture four or five hours before milking and that they be fed alfalfa hay immediately after milking, to entirely avoid feed flavors from those sources. Cooling is ineffective and aeration only partially effective in eliminating alfalfa flavor.

Beck (17) observed that rye pasturage produced a more offensive flavor and odor in milk than did wheat pasturage.
The undesirable flavor was greatly reduced, or eliminated, when the cows were removed from pasture four hours before milking. Aeration of the milk was non-effective in removing these odors.

According to Lindsey, Holland, and Smith (95) feeding of distillers' or brewers' dried grains in limited quantities does not injure the flavor of milk. The feeding of beet pulp slices, beet tops, dried beet pulp, and mangels produced no bad flavors in milk, according to Reece (125), Trout and Taylor (160) and Hening and Dahlberg (79).

California workers (134) found that the usual concentrates when fed one to two hours before milking in normal quantities did not give milk sufficient feed flavor to make it undesirable to the average consumer. Wheat bran seemed to improve the milk flavor when fed in quantities of 0.5 to 7.0 pounds one hour before milking.

Lindsay, et al., (93) fed 2.3 pounds of soybean meal and Rosengren (139) fed 5.5 pounds soybean cake daily with no resulting undesirable flavors in the milk or butter. Likewise, Olson (113) and Goodale (65) noticed no appreciable effect on the flavor of milk and milk products when soybeans constituted 25, 50, or even 100 per cent of the grain mixture.
Nevens and Tracy (112) used three groups of eight cows each in a study of the effect of ground soybeans and different quality soybean hays on the flavor and composition of milk and butter. One group was fed the regular herd ration throughout the experiment, while the other groups were reversed on the various experimental rations. The experimental periods were one week in duration. The ground soybeans, when fed, formed 10 to 25 per cent of the grain mixtures. The maximum amount of soybeans fed was 2.5 pounds per head daily. Results of the experiment showed that neither good quality, nor poor quality soybean hay had effect upon the flavor of the milk (raw or pasteurized), skim milk, cream, or butter. Ground soybeans were likewise without effect upon the flavor of the milk or milk products. The most pronounced effects of the ration were upon the body of the butter. Ground soybeans caused the butter to be gummy and the condition became worse when the proportion of soybeans in the ration was increased. Soybean hay had a similar effect but to a less degree. It is doubtful as to whether or not the experimental periods were sufficiently long for the results to be conclusive.

Williams, Cannon, and Ispe (168) report that cows yielding as much as 50 pounds of milk daily were successfully carried over a period of 22 weeks on silage, fed ad
libitum, and soybeans, fed at the rate of one pound per five pounds milk produced. The silage was made from corn silage, sorghum silage or sorghum silage supplemented with one pound corn per 20 pounds of silage. Silage consumption averaged about six pounds per 100 pounds liveweight. Up to nine pounds daily of soybeans were consumed by a single cow. The experimental ration of soybeans and silage caused no marked changes in milk flavor.

Another flavor study was made at the Iowa Station by Chin (32). When soybeans and linseed meal constituted 11.1 per cent of the concentrate mixture fed to dairy cows no objectionable flavors in the milk resulted when the milk was collected and held in glass or tin containers, even when the latter were badly corroded.

Babcock (8, 9, 10, 12, 13) and Gamble and Kelly (55) found that when fed to dairy cows one hour before milking, cabbage, turnips, rape and kale noticeably affected the flavor and odor of milk; whereas green rye, green cowpeas, potatoes, dried beet pulp and carrots affected milk only slightly. Green corn, oats, peas, soybeans, pumpkins and sugar beets had practically no effect. Feeds which were noticeably effective in causing objectionable flavors, produced off-flavors in the milk when as little as 15 pounds were consumed one hour before each of the two daily milk-
ings. These objectionable flavors and odors were accentuated when 30 pounds of these feeds were consumed twice daily. With most of the feeds studied the consumption of 30 pounds immediately after milking had practically no deleterious effect on the milk produced at the next milking. With cabbage and turnips, even though fed immediately after milking, slight flavors were occasionally found in the milk obtained at the next milking.

Babcock (15), Roadhouse and Henderson (135) found that the feeding of green barley, wild oats, foxtail, and filaree two hours before milking, in quantities required for satisfactory nutrition and as a sole source of roughage, imparted undesirable feed flavors in milk, varying from slight to strong.

Babcock (6, 9, 10, 12, 13) and Gamble and Kelly (55) agree that feed flavors are more pronounced in the cream than in the milk from which the cream is taken, and that proper aeration reduces strong, and may eliminate mild flavors and odors in milk caused by feeding highly flavored feeds.

The bitterweed frequently found in southern pastures, was discovered by Babcock (14) to be an exception to the usual rule that feed flavors are more pronounced in cream than in the milk from which the cream is taken. This flavor was more pronounced in skim milk than in whole milk.
or its cream. An exception to the rule that feed flavors are not imparted to milk except for a few hours after feeding was also noticed for when cows consumed ten pounds of bitterweed the flavor was evident in milk produced 24 hours later. The milk produced 27 hours later was practically free of this flavor.

Combs (33) states that garlic, French weed (stink grass), rag weed, pepper grass, wild onion, and many other weeds impart off-flavors to milk. According to him, the causative factor in French weed is due to an enzyme, myrosin, which hydrolyzes the glucoside, sinigrin, in the seed, into mustard oil and other flavoring compounds, one of which may be oil of garlic. He showed that the maximum flavor effect was present in milk from cows producing 30 pounds daily when they were fed 30 grams of French weed seed three hours before milking, and that by the end of five hours the milk flavor was practically normal. Since cows do not relish French weed seed, or the green plant, such obnoxious weeds will not be eaten if the cows are fed a good grain and roughage ration.

Babcock's experiments (11) with garlic indicate that feed flavors enter milk mainly through the body of the cow. Garlic flavor and odor were detected in the milk as soon as one minute after ingestion. During a ten-minute period, the intensity of the garlic flavor increased as the time inter-
val between feeding the garlic and taking the milk samples increased. At the end of the ten-minute period, the flavor intensity began to subside. Milk from cows that consumed one-half pound of garlic four hours before milking possessed a garlic flavor to a very objectionable degree. Milk drawn after an interval of seven hours was practically free from the noxious flavor. An inhalation of garlic odor by cows for ten minutes produced a strong garlic-flavored milk within two minutes, which practically disappeared after 90 minutes. The detection of a very strong garlic odor in the blood within 45 minutes after feeding two pounds of garlic tops indicates that the flavor is transmitted by the blood to the udder.

MacDonald and Crawford (100, 101) state that the flavor and odor of "onion milk," for the most part, is due to unsaturated organic sulfur compounds, such as allyl sulfide and disulfide, which are produced by members of the onion and garlic family. The volatility of these compounds at moderate temperatures is taken advantage of in removing them.

It has also been demonstrated that undesirable flavors in dairy products may result from the absorption of foreign odors after the milk has been produced.
Alt (1) demonstrated an increase in the ability of milk to absorb nitrogen gas up to 50°C, above which the nitrogen absorbed by the milk decreased. In skim milk with 0.12 per cent fat there was no increase in the nitrogen absorption associated with increased temperature, but whole milk containing 3.65 per cent fat increased in its nitrogen content up to 35°C. Further increases in the absorption of nitrogen by the milk were found to occur with milk containing 5.7 per cent fat and with butter containing approximately 82 per cent butterfat. Thus, it was concluded that the fat of milk is largely responsible for the qualities which make dairy products so susceptible to the absorption of foreign flavors and odors.

Russell (140) reported that when milk is exposed during the milking process and very often after its withdrawal to an atmosphere that is liable to contain odors of an undesirable character, it may thus contract flavors by direct absorption.

As a result of trials in which corn silage was spread on the floor underneath two cows in a stable with the doors and windows tightly closed, Gamble and Kelly (55) report that silage-tainted barn air may have some effect on the flavor and odor of milk under such extreme conditions.
They concluded that the effect would be relatively slight under average stable conditions, where some ventilation exists.

In support of this statement Farrington (53) states that the success of feeding silage depends almost entirely upon keeping the stable air free from silage odor.

2. Oxidized Flavor

Since the early days of the dairy industry tellowy, oily and fishy flavors, which are considered to be caused by oxidation, have been observed in storage butter. Many of the factors which favor oxidative changes in butter are concerned also with the production of off-flavors in milk, dried milk, ice cream, and condensed milk. The immensity of this subject necessitates limiting this review primarily to those factors which are directly related to the experiment.

In rather recent work, Anderson et al., (4, 6) advance the following theory relative to vitamin A and milk flavor:

The oxidized, rancid, flat, and insipid flavors which develop in milk of low bacterial content have their origin in a carotene deficient ration. This affects the milk directly by reducing its carotene content, and what appears to be more important, causes a vitamin A deficiency in the cow. The
resulting abnormal conditions in certain tissues lead to an abnormal distribution of enzymes in blood and milk. Certain other substances, which are not normally present in milk, may find their way into it. In this way we account for the excessive amounts of lipase which are present when milk develops a rancid flavor. This theory is in harmony with present knowledge concerning the function of vitamin A in maintaining the integrity of epithelial tissue. The secretory tissue of the mammary gland is derived from epithelial tissue. Liver tissue is especially high in vitamin A, consequently, any deficiency of this vitamin could be expected to result in an abnormal distribution of enzymes having to do with fat metabolism. Furthermore, the manner in which off-flavors gradually develop on deficient rations and are rapidly eliminated on a carotene high ration is characteristic of vitamin responses.

Thurston (157) proposes the following classification for milks as regards the development of an oxidized flavor therein: (1) Spontaneous milk - that capable of developing oxidized flavor spontaneously, i.e., without the presence of iron or copper as a contaminant; (2) Susceptible milk - that which does not develop oxidized flavor spontaneously, but is susceptible in the presence of copper or iron; (3) Non-susceptible milk - milk which will not become oxidized
even when contaminated with iron or copper.

a. **Composition of the milk.** Barkworth (16) suggests that since some workers believe the enzymes in milk serum to be heat labile they may be involved in the development of oxidized flavor. The basis of this theory is that milk heated to very high temperatures develop an off-flavor. Dahle (40) reports that heating milk to 145°F. for 30 minutes increased the tendency for oxidized flavor to develop, while heating to 170°F. inhibited it. However, Gould's results (66) do not support the above theory as he was able to develop oxidized flavor in milk heated to 78°C. for 30 minutes.

Roland and Tebler (139) report an apparent relationship between the copper-induced oxidized flavor and the fat content of milk, as each increase of about one per cent fat in whole milk was detected by a significant decrease in the flavor score. Skim milk exposed to large areas of copper developed a metallic flavor but never an oxidized flavor.

Oxidized flavor in milk and cream is generally accepted to be the result of oxidation of one or more of the lipids present in milk. The oxidation of the unsaturated fatty acids present in butterfat was originally thought to be the source of oxidized flavor, but the evidence presented showed that phospholipids may play an important role in the development of oxidized flavor in milk and cream.
Horrall (88) found that as the amount of butterfat in milk increased so did the amount of lecithin present increase. This finding seems significant since Thurston, Brown, and Dustman (155) present evidence which indicates that the phospholipid fraction of susceptible milk rather than the butterfat was the substance that underwent oxidative changes. They showed that the intensity of the oxidized flavor in cream, skim milk, buttermilk, and butter obtained from oxidized milk was in direct relation to their phospholipid content. In a later work (146) they explained the inhibiting effect of agitation of cold milk on the development of oxidized flavor as being the result of partial transfer of lecithin from the fat globule surface to the plasma. They considered this as further evidence that lecithin was the constituent affected.

Brown, Dustman and Thurston (24) in 12 trials during the winter months of two successive years found no measurable change in the iodine number of milk fat from "oxidized" and normal milk. In a more recent work Swanson and Sommer (149) likewise were unable to find any difference in the iodine number of butterfat from normal and oxidized flavored milk. However, they found that the development of oxidized flavor is accomplished by a marked decrease in the iodine number of
the phospholipid fraction. Dahle and Palmer (42) substantiated the findings of Thurston et al. (155) in regard to the development of oxidized flavor in susceptible milk, but they found that on the addition of washed cream to skim milk from spontaneous milk that the remade milk developed an oxidized flavor. Contradictory to this, Chilson (31) found that on the addition of washed cream to skim milk from spontaneous milk that the remade milk would not develop an oxidized flavor. In support of these latter findings Roland and Trebler (138) found a marked decrease in copper-induced oxidized flavor in reconstituted milk. They suggest that the removal of lecithin or related substances by the separator or changes in their distribution between the fat and aqueous phase may be responsible for the decreased sensitivity.

b. Feed and season of the year. One of the earliest reports dealing with the relation of the feed given the cow, and season, to the development of oxidized flavor in milk was made by Kendle (91). He reported that oxidized flavor seems to occur only during the winter and also that green feed as well as the fresh hay produced from it contain considerable amounts of reducing substances which tend to prevent oxidized flavor development, or lessen its intensity. In support of these findings several investigators (23, 42, 67, 91, 147) have reported that green feed contributes
substances to milk which inhibit the development of oxidized flavor.

Extensive investigations have been made in an effort to determine the nature of the inhibiting substances carried in green feeds. Ritter (131) and Chilson (31) found the first clues as to the probable nature of these substances in 1935 when they prevented the development of oxidized flavor by the addition of ascorbic acid to the milk. These findings have been verified by other workers (18, 25, 40).

Brown and coworkers (23, 35) also maintain that ascorbic acid is helpful since in their experiments the susceptibility of milk to oxidized flavor was greatly reduced by feeding one quart of lemon or tomato juice to each cow daily. Similar results were obtained through feeding one-half gram of pure crystalline ascorbic acid daily per cow. This occurred in spite of the fact that ascorbic acid rapidly disappears in the presence of bacteria, untold numbers of which always exist in the rumen. The vitamin C value of milk fluctuates quite a bit even though its vitamin C content is not significantly altered by the amount of vitamin C in the ration. Garrett, Arnold, and Hartman (56) obtained a significant correlation (over 0.6) between the vitamin C content and the flavor of milk used in their study. They recommend that special efforts be made to preserve ascorbic acid in milk since it appears to have certain reducing prop-
erties which exert protective action on milk.

In a later work Garrett et al. (59) studied the effect of succulent roughages on the flavor of milk and their stabilizing effect on ascorbic acid in milk. They found that the feeding of properly ensiled molasses grass silage has a greater stabilizing effect on ascorbic acid of milks in storage than does corn silage or beet pulp. This stabilizing effect tended toward milk of better flavor. However, Gjessing and Trout (71) point out that the rapid reduction of ascorbic acid does not always imply oxidative changes in milk for this offensive flavor failed to develop in some of their samples which contained no ascorbic acid after 3 days storage, even though copper was present.

Whitnah, et al. (16b) found no relationship between the amount of vitamin C and the development of oxidized flavor in milk from cows of the same breed. These findings appear to be somewhat at variance with the findings of other workers (18, 22, 25, 40, 56, 59).

In addition to the stabilizing effect of vitamin C or ascorbic acid on the flavor of milk Garrett et al. (57, 58) also point out that the carotene of the ration has certain reducing properties which exert a protective action on the milk. In support of this Beck et al. (18) and Brown et al. (35) found that a carotene supplement to a cow's ration quickly reduces the incidence of oxidized flavor. These
findings agree with those of earlier reports (3, 4, 5).

In their study of the effect of succulent roughages on the flavor of milk, Garrett, Arnold and Hartman (60) point out that legume and grass silages, when properly made and fed, produce milk with higher yellow color, finer flavor and greater resistance toward the development of oxidized flavor than do corn silage, beet pulp or dried citrus pulp. Alfalfa silage is almost equal to spring pasture in endowing milk with yellow color and is equal to, or better than, pasture in producing milk of fine flavor and high resistance toward oxidized flavor development. This and other work (3, 4, 5, 18, 25, 28, 57, 58) point to the favorable effect of vitamin A or carotene in producing milk with a better flavor and more resistance to the development of oxidized flavor.

Another angle relative to the effect of feed on the susceptibility of milk to oxidized flavor has been presented by Henderson and Roudhouse (76). They state that if cows are fed poor rations, which necessitate their drawing on body fat, the milk produced will be more susceptible to the development of oxidized flavor, due to an increased percentage of the unsaturated fatty acids in the butterfat. It would appear that any condition or feed that will greatly increase the degree of unsaturation of milk fat will increase the susceptibility of the fat to become oxidized. The findings of Stebnitz and Sommer (147) that the stability of the fat was
influenced by its unsaturation is in accord with those of Henderson and Roadhouse (76).

In a study to determine the relationship of oxidized flavor to the percentage of unsaturated fatty acids in butterfat, Corbett and Tracy (35) fed two groups of three cows each one-third of a pound of oil daily for three days, then two-thirds pound daily for three days, after which they were fed one pound daily for six days. Either corn oil or coconut oil was fed alternately to each group throughout the experiment. The control ration consisted of alfalfa hay, corn silage and a grain mixture. Results of the experiment showed that the susceptibility of milk to the development of oxidized flavor was not correlated with the iodine number in the range of 24 to 40. However, milk with an iodine number greater than 40 developed an oxidized flavor slightly more frequently than milk having a lower iodine number. It seems reasonable to conclude that the reasonable nature of the occurrence of oxidized flavor cannot be explained on the basis of changes in the iodine number of the milk fat.

Brewitt and Parfitt (12), studying effects of feeds on oxidized flavor in pasteurized milk, fed rations which included ground white and yellow corns, ground oats, ground soybeans, wheat bran, soybean oil, soybean oil meal, linseed oil meal, dried brewers' grains and gluten feed. The milk
samples were placed in aluminum pails soon after milking, pasteurized in Pyrex flasks, and then cooled to 4.4°C. Oxidized flavor failed to develop in any of the samples from cows fed these rations when stored at 4.4°C. for 72 hours. To determine their effect on the development or retardation of oxidized flavor, minute quantities of copper compounds were added to the samples of milk. The results indicate that the rations containing soybean oil, as such or as unprocessed beans, tended to produce milk which, upon holding, developed less oxidized flavor than did the milk from cows fed the other rations.

In a similar study Brown, Dustman, and Weakley (27) placed on a low fat ration eleven cows whose milks were susceptible to oxidized flavor when contaminated with copper. After a preliminary period of a few weeks the cows were divided into two groups; group I received one pound of oil supplement daily and group II served as the control. Coconut, soybean, and hydrogenated soybean oils were fed. In contrast to the other oils, one pound of the soybean oil when fed daily, greatly increased the iodine number of the butterfat and thus increased the susceptibility of the milk to metal induced oxidized flavor. These results are contradictory to those of Previtt and Parfitt (132) already referred to. However, it is difficult to make a fair comparison be-
between the results since Previtt and Parfitt do not give the amount of oil supplement used in their experiments.

Dahle and Carson (39) report that cows fed alfalfa hay produced milk more susceptible to oxidized flavor than cows on other roughages. Likewise, Henderson and Roadhouse (75) found that three cows produced a fat more stable in nature when maintained on an oat hay ration than when alfalfa hay was fed. No significant difference in the rate of oxidation of the fat when animals were maintained on the dry and green alfalfa regimes were observed.

Evidence has been presented, which seems to indicate that there is some relationship between the susceptibility of milk to the development of oxidized flavor and the percentage of unsaturated fats in the milk. However, under normal feeding conditions, it is doubtful as to whether or not the percentage of unsaturated fats would be increased sufficiently to produce milk with an increased tendency to develop an oxidized flavor.

c. Age of the cow. Many workers consider the age of the cow in the production of oxidized milk of less importance than other factors. This view is sustained by Guthrie and Brueckner (69) who found no apparent relationship between age of cows and the occurrence of oxidized-flavor in their milk. Contrary to this, Hening and Dahlberg (86) compared
the milk from six first calf heifers with that from six mature cows. Of 58 samples of pasteurized milk from the heifers scored the first day after it was pasteurized 6.8 per cent developed an oxidized flavor, whereas none of the 57 samples from the cows developed an off-flavor. A comparison of the same milk on the third day after pasteurization showed 24.5 per cent oxidized for the heifers and 12.0 per cent for the cows. On the third day after pasteurization a comparison of this same milk with 0.25 p.p.m. of copper added showed that 54.4 per cent of the milk from the heifers and 79.3 per cent from the cows had an oxidized flavor. While this experiment was on a small scale, definite evidence is presented which indicates a close relationship between age and the incidence of oxidized flavor in milk.

In a study of oxidized flavor in the milk of individual cows Corbett and Tracy (34) collected five hundred and thirty-eight samples from 136 cows over a period of six months. Of the metal-free milk samples, 1.48 per cent and 10.01 per cent had an oxidized flavor after 1 and 3 day holding periods, respectively. Of the samples to which 1 p.p.m. copper was added, 78.9 per cent and 91.7 per cent had an oxidized flavor after 1- and 3- days storage periods, respectively. The data were analyzed to determine the
correlation between oxidized flavor development and breed of cow, age of animal, period of lactation, milk and fat yields. Comparison on the relationship of age of cow to occurrence of oxidized flavor revealed that two- and three-year old cows gave milk (1 p.p.m. Cu added) which developed oxidized flavor to a greater degree than that from older cows. The greatest incidence of oxidized flavor development in a metal-free milk held three days was also in that produced by cows under 5 years of age. The averages were 10.10 per cent for the younger cows and 4.27 for the cows 5 years or older.

Evidence (34, 69, 80) has been presented which points rather conclusively to a very close relationship between age and the occurrence of oxidized flavor in milk. This seems to be especially true of first calf heifers.

d. Breed of the cow. Very little work has been reported on the relationship of breed of cow to the incidence of oxidized flavor in milk.

One of the earliest experiments concerning this relationship was performed by Guthrie and Brueckner (69). They examined samples of milk from 150 cows in five herds after it was pasteurized and stored in brown glass bottles. Approximately 10 per cent produced milk which developed only slight oxidized flavor. No relationship could be established between breed and the development of oxidized flavor in the
Using small groups of cows from each breed Stebnitz and Sommer (147) observed that the milk fat from Holstein cows on winter rations was more susceptible to oxidation than that from the other breeds. The milk fat from the Guernsey and Ayrshire had an exceptionally high stability value.

Whitnah, Martin, and Beck (165), in a study to determine the relationship between carotene, lecithin, and vitamin C and the occurrence of oxidized flavor, also made some observations relative to the relationship between breed of cow and incidence of oxidized flavor. Samples of morning milk from the four breeds of dairy cows in the College herd (60-70 head) were collected in glass bottles on three consecutive days during the months of December to April inclusive. The oxidized flavor was detected in 12.7 per cent of the 922 samples examined during the regular monthly herd test. They found that the frequency with which oxidized flavor developed in the milk of the different breeds was in the order (starting with the highest), Holstein, Ayrshire, Guernsey and Jersey. This report contradicts that by Guthrie et al. (69).

In a later experiment these same workers (16) examined 1127 samples (60-70 cows) of raw milk held 3 days at 40°F.
and found an oxidized flavor in 11 per cent of the samples. Oxidized flavor occurred in 6.1 per cent of the Jersey samples, 7.8 per cent of the Guernsey samples, 15.5 per cent of the Holstein samples and 19.4 per cent of the Ayrshire samples. This report differs from their previous one (165) in that the Holstein and Ayrshire breeds are revered with respect to the frequency with which oxidized flavor developed.

In contradiction to the work done by Whitnah et al. (18, 165) and Stebnitz and Sommer (147) and in agreement with that done by Guthrie and Brueckner (69) is a rather recent report by Corbett and Tracy (34). They studied the incidence of oxidized flavor in the milk of individual cows within one herd. More of the details of their methods of experimentation are included under section c."Age of the cow." Their comparisons revealed little difference between the various breeds as far as occurrence and development of oxidized flavor are concerned, though the Ayrshire breed seemed somewhat less susceptible.

Evidence has been presented which indicates that breed may (18, 165, 147) or may not (34, 69) be a factor in the extent of oxidized flavor development. Since carotene when added to milk definitely inhibits the development of oxidized flavor (and vitamin A does not) it seems quite logical that milk with a naturally high carotene content should be less susceptible.
e. Miscellaneous. Thus far the review of literature has been concerned primarily with flavor factors which are affected by treatment of the cow in the production of milk and which have a more or less direct bearing on the results of this experiment. Needless to say, there are numerous other causes of oxidized flavor in milk and cream which have not been mentioned. The grouping of these factors under this heading does not in any way reflect on their importance relative to the occurrence of oxidized flavor in milk. On the contrary, they are probably as important or more important than any of the factors already reviewed and should receive due consideration when trying to produce milk of a high quality. However, it is felt that since they have little bearing on this experiment, it is only necessary to point out their significance with respect to the development of oxidized flavor.

The occurrence of most oxidized flavors in dairy products, especially milk and butter, after the milk is produced, seems to be related primarily to the presence of oxidizable metals. In order for any metal to corrode or to affect the dairy product, it must be capable of entering into solution. Several factors seem to influence the effectiveness of metals in catalyzing oxidative processes, namely, (a) the kind of metal, (b) the temperature of milk at time of contamination, and (c) the relation of metal contamination to pasteurization.
The metals effective in causing oxidized flavors are mainly iron, copper and copper alloys. Probably the first to attribute oxidized (oily) flavor in butter to iron contamination was Seigman (164) in 1891. Later others (72, 90) noted this same relationship while Rice (130) found a distinct oxidized flavor in sweetened condensed milk which developed during its processing in copper oxide-coated pans.

Golding and Seigman (64) were probably the first workers to report the effect of copper on the flavor of milk. Since that time the relationship of copper and iron to the development of oxidized flavor in milk and cream has been noted by a large number of investigators (129, 43, 64, 68, 104, 146, 150, 154). In many of these studies this flavor has been referred to frequently as cappy, cardboard, oxidized, papery, metallic, smery, oleaginous, and tallowy. It is the consensus of opinion among most investigators that this flavor is the result of oxidation and that the term "oxidized" is the most appropriate.

In an effort to explain the action of copper in producing oxidized flavor in milk, Olson, Brown, and Carson (114, 115) have recently made a very interesting, rather recent report. They point out that the development of oxidized flavor in milk seems to be closely associated with the ionization of copper and its destruction of ascorbic acid. Apparently
anything which decreases the ionization of copper will in

turn retard the destruction of ascorbic acid and in this

manner tend to retard oxidized flavor development. These

workers showed that citric acid, ascorbic acid, and amino

acids form copper complexes with copper and thereby decrease

the ionization of copper.

Numerous factors involved in the processing of milk and

cream have been shown to be related to the production of oxili-
dized flavor, primarily through catalytic action on metals

with which the milk and cream come in contact. Only a few

of the more important ones will be mentioned.

Temperature was one of the first factors shown to pro-
duce a catalytic effect on metals and thereby an increased
tendency for an oxidized flavor to develop in milk and cream.
Rice and Miscall (129) showed that less copper was dissolved
at boiling temperature than at room temperature. Though all

investigators do not agree as to the temperature of milk and

cream which favors the greatest corrosion of metals, many

(61, 62, 63, 123, 129, 159, 166) have demonstrated that
temperature plays an important role in this respect.

Though probably not as important as temperature, the

presence of oxygen is another factor which has been shown
to increase the rate of corrosion, whereas the presence of

carbon dioxide decreased it (61, 62, 63, 108, 129).
One of the most important factors which causes the development of oxidized flavor in dairy products and with which the dairy industry has been quite concerned is light. As early as 1894 Von Ilecki (161) observed that butter must be kept away from direct sunlight and at a low temperature to maintain its quality. The deteriorating effect of light on milk was brought to the attention of investigators as early as 1807 by Burr (29). In 1930 Hammer and Cordes (72) found that a rancid flavor developed in milk when exposed to sunlight in plain glass bottles. Various other workers (22, 26, 67, 70, 73, 76, 146, 150, 172) have proved that light has an important accelerating effect on fat oxidation.

Other factors, such as bacterial action (52, 91, 137, 151, 152, 155, 156) and anti-oxidants (41, 4', 131) have been shown to be interrelated with the development of oxidized flavor in dairy products.

It seems to be the consensus of opinion among most investigators that of all the factors concerned in bringing about oxidative changes in dairy products metallic contamination, particularly by copper, is the most important at present.

3. Rancid Flavor

This flavor is usually characterized by a bitter flavor and a rancid odor, suggestive of butyric and other low molecular
weight fatty acids (47).

Lipase activity is generally regarded as one of the causes, if not the sole cause, of natural rancidity. In 1922, Rice and Markley (128) revealed that lipase, a term reserved for those lipolytic enzymes which split the natural neutral fats, is normally present in milk. Since then other workers (51, 82, 86, 93, 92, 121, 124) have verified the presence of lipase in milk. Palmer (119) demonstrated a type of bitter milk (rancid milk), which he associated with advanced lactation and with the difficulty of churning cream produced from late-lactation milk. He related this difficulty to the formation of soaps which accompany the liberation of free fatty acids during lipase fermentation. Little (97) showed that the milk produced by certain cows after eight months of lactation had a higher pH, per cent of chlorides and cell content than the milk that the same cows produced during the first four months of the same or succeeding lactation periods. These results would tend to favor Palmer's (119) hypothesis.

Contrary to Palmer's results, Roahen and Sommer (136) failed to note a relationship between lipolysis and stage of lactation in their study of milk from 18 cows at known stages of lactation. In support of Roahen and Sommer's (136) work Herrington and Krukovsky (65) made a study of
lipolysis in the milk of 61 cows in which they found a great deal of variation in lipolytic action among the milks of individual cows. They were unable to obtain a correlation between the rates of lipolysis and the stage of lactation, stage of gestation, or the milk production of the individual cows. The results of this work are contradictory to those reported by Palmer (119).

In this connection, Anderson (3) and Anderson et al. (5) state that the cows used in their study produced milk free of rancid and oxidized flavors when machine-cured alfalfa hay was fed, whereas the milk produced when poor field-cured alfalfa hay was fed developed either an oxidized or rancid flavor. These observations were made during the autumn, winter, and spring on two large farms. Oxidized flavors as well as rancid flavors were eliminated by the addition of carrots to the ration. The factor, or factors, responsible for the production of good-flavored milk appeared to be present in high quality carrots to a much greater extent than in machine-cured alfalfa.
B. Factors Related to the Chemical Constants of Butterfat and the Body and Consistency of Butter

1. Character of the Ration

Evidence has already been presented that feeds, as well as many other factors, may exert a pronounced effect on the flavor and quality of milk. Likewise, it has been known for some time that feeds (50, 58) may also have a noticeable influence on the chemical constants of butterfat.

As it is evident that the fat content of a feed is the primary constituent which effects the chemical constants of butterfat, it might be well to mention briefly some work concerning the relationship between food fats and milk fats. Holland et al. (87) state that neither proteins nor carbohydrates have any appreciable influence in changing the chemical compositions of the butterfat. On the other hand, different oils and fats do modify, to an extent, the chemical composition of butterfat, the modification depending upon the composition of the oils and fats fed.

Relative to the normal composition of milk fat, Hilditch and Jasperon (82) state that while oleic acid is the chief unsaturated acid in butterfat, six others may be present in small proportions, together with nine saturated acids of
which palmitic acid is present in largest amount, although there are appreciable amounts of butyric, myristic, and stearic acids. In this connection, Peterson, Palmer, and Nickles (20) point out that the modification of milk fat by the diet occurs in opposition to the normal tendency of the gland to secrete a product of constant composition. They theorized that the blood precursor of milk fat is first transformed into glandular fat, intermediate in character between body fat and milk fat.

Considerable work concerning the influence of food fats upon blood lipids and milk fats has been conducted by Maynard, McCay, and Hadsen (106). In one experiment, the iodine numbers of the blood lipids and the milk fats were followed when cows were fed rations containing fats having a low and high iodine number. In one-half of the cases a marked change in the iodine number was noted in a composite of the milk secreted during the first 16 hours following a change in ration, in all cases within the next 24 hours, while the maximum change was attained within three or four days. The data indicate that the secretion of milk fat in lactation must pursue a very direct course from food fat to milk fat or that the various processes involved in an indirect course must take place very rapidly. This work is in agreement with that of earlier workers (50, 89, 110) that the food eaten by the cow, especially when the oil content is high, is closely
related to the quality of fat produced.

Soybeans and cottonseed products were among the first protein feeds selected for study because of their economic importance as well as the obvious changes in the character of butter which accompany their use in excessive amounts.

As early as 1894, Brooks (20) reported that butter made from cream produced by cows fed soybeans was of a higher color and much more desirable in texture and flavor than that made from cream produced by cows fed a cottonseed ration. Otis (116) and Lindsay, et al. (96) agreed that the feeding of soybeans and soybean meal produced a soft butter if they constituted as much as 50 per cent of the grain mixture. Otis considered it possible for a dairymen to obtain butter of any consistancy by regulating the proportions of cottonseed meal and soybean meal in the ration. Olson (113) at the South Dakota Station concluded that even when soybeans constituted 75 to 100 per cent of the grain ration, the effect on butter is not easily detected by inspection. On the other hand, Goodale (55) reports that when soybeans constituted one-half of the grain mixture, the butter became so soft that great care was necessary to keep from over-working it, and ill-effects were produced when the beans constituted only one-fourth of the mixture. A probable explanation for the differences in results obtained by these workers may be the
region and climatic conditions under which the experiments were conducted.

Work by Williams (167) at the Iowa Station supports previous investigations (65) that soybeans will produce a soft, almost colorless butter if fed in excessive amounts. By comparing the iodine values of the soybean oil and the butterfat he concluded that when cows consumed as much as 1.5 to 2 pounds of soybean oil daily, the effect of the oil on the milk fat was the same as if 23 per cent of it had been mixed with the original butterfat. The effect was just about one-half as great when 2.7 pounds of soybeans were fed. He also pointed out that when soybean oil was fed without extraction from the soybeans there was a decrease in milk yield, and an increase in butterfat production, while when fed as free oil, the effect on yields of milk and fat were in the opposite direction. When the free oil was fed the iodine and thiocyanogen numbers were at their maximum and the Reichert-Weissland Polenske values were at their minimum. The oleic acid content was highest during the oil feeding and the linoleic and during the soybean feeding periods.

As early as 1897, Moore (109) detected no deleterious effect on the composition of the butterfat when cows were getting as much as 5 pounds of cottonseed meal or 6 pounds of cottonseed per day, if fed in combination with corn silage,
hay and bran. In later work, Cockles and Palmer (31) found that cottonseed products when fed to cows decreased the saponification and Reichert-Weissl numbers and increased the iodine number of the butter. The butter frequently had a firm, gummy body, a flat, oily taste and the ability to withstand relatively high temperatures without losing its body. Their suggestion regarding the improvement of the keeping quality of butter by feeding cottonseed products is as follows: They stated that as much as 5 pounds of cottonseed meal may be fed daily with a liberal ration of corn silage without seriously affecting the market quality of the butter. With the dry feeds, about 3 pounds daily would be a liberal cottonseed ration if the production of high grade butter is at stake. In a recent report Miller and Wise (107) found that the feeding of cottonseed meal as the only concentrate compared with feeding a normal grain mixture decreased the saponification number and increased the iodine number and Refractive Index of the butterfat.

Neasham and Gelpi (111) state that a butter of fairly normal consistency can be produced when cottonseed meal constitutes 30 per cent of the concentrate mixture. However, 40 per cent cottonseed meal may produce butter of abnormally hard consistency, especially when fed with dry foods. Green pasture and good corn silage may counteract somewhat the
effects of cottonseed meal on butter quality. The counteracting effect of green pasture may be explained by the work of Dean and Hilditch (45) and Hunzinker (69). They found that when cows go on pasture there is an increase in the oleic and linoleic acids of the fat secreted.

Because most of the experiments had been conducted on unbalanced and uncommonly used rations, Ovarmann and Garrett (117) studied the influence of certain balanced rations on the chemical and physical properties of milk fat. Three groups of cows were fed special grain mixtures, so composed that 50 per cent of the total protein of the mixture was supplied by either cottonseed meal, linseed meal or ground soybeans. Corn silage and good quality alfalfa hay were fed liberally throughout the experiment. The results show that the differences between the fats produced on these different rations were not large enough to be of particular interest.

Among the more recently reported work on this subject is that by Herzer et al. (33). They found that cottonseed hulls produced a low-scoring butter, which acquired a very noticeable firm, gummy texture. Soybean meal counteracted considerably the firm, gummy texture produced by the feeding of cottonseed meal. Finally these workers state that quite desirable effects on the texture of butter may be obtained by the proper proportioning of soybean and cotton-
seed meals in the dairy ration.

Hill and Palmer (85) list the following points relative to the effect of certain feeds on the properties of milk fat and butter: (1) when barley constitutes 35-50% of the digestible nutrients of a low fat ration, along with alfalfa or timothy hay, a hard butterfat with a low iodine value is produced; (2) when oats or corn are substituted for 35-50% of the digestible nutrients of a low fat ration, containing alfalfa hay, the physical characteristics of the butter produced are satisfactory from the market standpoint; (3) when 0.6 pound or more of linseed meal is fed daily the butterfat produced shows a significant increase in the content of fatty acids less saturated than oleic acid.

Cranfield (37) feeding cocomoat cake and linseed cake, and Cranfield and Taylor (38) feeding linseed cake and hemp-seed cake, found that when cows were removed from poor pasture to well balanced rations containing these products a considerable rise in the Reichert-Meissel, Kirschner, and Polenske numbers and a fall in the refractive index of the fat occurred. Work relative to the effect of roughages on the fat constants has been reported by Herzer, Moore, and Cowsert (83). They set forth the following conclusions: (1) Johnson grass, Sudan grass, Bermuda grass, soybean and lespedeza hays, when fed to cows, may produce butter with a flavor and texture
equivalent to that produced on alfalfa hay; (2) the fat constants of the butters produced from these hays were not significantly different from those of the butter produced from alfalfa hay, with the exception of lospedeza, which produced a higher iodine number; (3) the cows fed sweet potatoes yielded butter that was firmer and harder than that produced when sorghum silage was fed. However, this butter, with an improved flavor, cleaned up quickly and left no trace of gumminess.

In general, the feeding of starches and carbohydrates have an opposite effect on the butterfat constants than do protein feeds rich in oils. Runziker, et al. (89) state that feeds rich in vegetable oils, as well as blue grass pasture, produce a soft butter relatively high in olein and low in volatile fatty acids, while feeds rich in starches and sugars and poor in vegetable oils tend to produce opposite effects.

Amberger (2) observed an appreciable lowering of the iodine number and an increase in the Reichert-Meissl and Polenske numbers when cows consumed beets. A substitution of sucrose for the beets produced similar changes, but not so great. Boes and Heyland (19) obtained a Reichert-Meissl number of 26.16, Polenske number of 6.16, saponification number of 234.2 and an iodine number of 24.22 when sugar
beets were fed exclusively. Similarly, Siegfeld (141, 142), Fritzscbe (54) and Luhrig et al. (99), in their study of beet leaves and beet tops, noted an increase in Reichert-Meissl, Polenske, and saponification numbers and a decrease in the iodine number and refractive index of the fat. According to Zaitschek (171), potatoes were similar to turnips in producing a fat having higher Reichert-Meissl and saponification numbers than when the cows were fed a dry feed.

2. Physical Conditions of the Cow

As early as 1916 Eckles and Palmer (49, 50) studied the influence of the plane of nutrition of the cow upon the composition and properties of milk and butterfat. Overfeeding and underfeeding experiments were conducted in which the planes of nutrition varied from normal to plus 104 per cent and from 15 to 70 per cent below normal. They discovered that underfeeding exerts a far greater influence on the composition and properties of milk and butterfat than does overfeeding. Underfeeding caused a marked reduction in the saponification and Reichert-Meissl numbers and an increase of about 15 units in the iodine number of the butterfat. Such effects appeared to reach their greatest intensity when the cow was held on about a 40 per cent subnormal plane of nutrition. Continuous underfeeding resulted in a partial
return to normal of the fat constants from the most abnormal values.

These workers point out that the state of flesh and the plane of nutrition of the animal previous to underfeeding apparently have no effect on the fat constants. On the other hand, the degree of underfeeding and the length of the underfeeding period appear to influence the fat constants. Data obtained by these investigators (49) warrant the general conclusion that normal milk and butter are to be expected when the cow is on a supernormal plane of nutrition as well as when the plane of nutrition is normal, providing there are no other influencing factors.

In support of the work by Eckles and Palmer (50) is a more recent report by Henderson and Roadhouse (76). Samples of milk were collected from two cows which were maintained on normal rations and then reduced to submaintenance rations of 12 pounds of alfalfa hay per day. The length of each regime was from 48 to 60 hours. Analysis of the butterfat revealed that the iodine numbers of the fats from the cows were increased about 17.3 units during the submaintenance regime.

Smith (144) and Smith and Dastur (143) have made some interesting studies relative to the effect of inanition on the blood lipoids and composition of milk fat. In one of the experiments (143) where feed was withheld from the cows
for a period of 12 days pronounced changes were produced in
the composition of the butterfat. The iodine number increased
about 10 units during the first day of the fast and then more
slowly to 16 units at the end of the period. The Reichert-
Meissl value decreased about 12 units during the first day
and then more slowly to approximately 16 units at the end of
the period. This increase in iodine number is in agreement
with that obtained by other workers (49, 50, 76) during
similar studies. Further analysis (143) revealed that the
chief change in the milk fat was a diminution of about 80
per cent in the original content of lower fatty acids up to
and including C₁₄, a deficiency which was compensated almost
entirely by an increase in oleic acid content.

Smith (144) was unable to explain the changes in the
chemical constants of butterfat in a study of the effect of
inanition on the blood lipoids of the lactating cow. No
changes in the lipoid content of the blood was found. As
an explanation, Smith and Dastur (143) point out that at
present there is no means of analyzing the triglyceride
fatty acids of the blood by themselves but only when mixed
with those originally present as cholesterylesters. There
is, therefore, every possibility that the triglyceride acids
of the blood may change and yet it may be impossible to de-
tect the change.
Brouwer and Martin (21) made an interesting observation on the influence of the injection of dinitrophenol on the flavor and composition of goat's milk which is similar to that made by other investigators (49, 50, 76). After the subcutaneous injection of this substance, a production of alkaline milk and an increase of about 15 units in the iodine number of the milk fat resulted. Because of the diminution in food consumption, due to a diminished appetite, and an analogous increase in the iodine value of the milk fat, it is suggested that the reserve body fat was utilized for the production of butterfat.

3. Climatic Conditions

It has been known (30, 44, 46) for sometime that dairy cows can withstand long periods of exposure to temperatures as low as zero degrees Fahrenheit with little loss either to production or in the efficiency of food utilization. However, temperatures in excess of 85°F. have a marked detrimental effect (127).

Other than seasonal effects, which have to do primarily with the types of feeds received by the animal, the effect that climatic conditions may have on the chemical constants of butterfat have received little scientific interest.
Hilditch and Sleightholme (84) observed a general change in the composition of butterfat, largely in the oleic acid content, which they attributed mainly to "winter conditions" (that is, either the change from outdoor to indoor conditions or from grass to indoor rations or both), and probably also to seasonal changes of temperature. These workers conclude that the influence of added fat in the diet is of a minor order as compared with that due to other causes.

The work of Dean and Hilditch (45) support that of the above workers (84). They point out that the seasonal rise in the iodine value of the fat is rather abrupt, the change being completed within two or three weeks after the cows have been changed to pasture. The degree of saturation of the winter fats may well be governed by at least three factors, namely, (a) an increase in saturation, relative to that of summer pasture milk fats, caused by either the indoor life or the indoor diet (or both), (b) the slow increase in unsaturated acid content due to increasing age of the animals, (c) a seasonal variation dependent upon the average temperature of a particular winter. Hilditch and Sleightholme (84) also noted that the degree of saturation of the fats produced in winter may be related to temperature.

In a later work Regan and Richardson at the California Station (126) report a study on the reactions of the dairy
cow to changes in environmental temperatures. A large psychrometric room was used in which the temperature was increased from 40 to 100°F. while air movement and relative humidity were maintained at the constant values of 50 feet per minute, and 60 per cent respectively. It was found that at 80 or 85°F. depending upon the breed, a pyrexial point was reached where the animals were no longer able to maintain heat balance. As the room temperature was elevated above this point, anorexia developed, milk flow declined, and an alteration occurred in the characteristics of the milk produced, which included a lowering of the casein and serum solids content, and an increase in the percentage of butterfat. The pH of the milk was raised, the freezing point was depressed, and a longer rennet coagulation time was required. The butterfat secreted was lower in volatile acids and higher in unsaturated compounds. These workers suggest that the changes in the characteristics of the butterfat secreted at high temperature may best be explained as the result of "hyperthermic undernutrition."

4. Stage of Lactation and Gestation

Eckles and Shaw (48) state that the fat constants are far less influenced by the breed and individuality of the animals than by stage of lactation. Their data imply that
the iodine number is rather high during the first month of lactation after which it subsides and remains at a fairly constant level until the fifth or sixth month, when it starts to increase. Likewise, Palmer and Isables (118) found that the fat of cow's milk during the later stage of lactation is characterized by a great depression of the saponification and Reichert-Heissel numbers and a great increase in iodine value and melting point. Kuhlman and Gallup (94) also observed that butterfat produced in the early part of the lactation period had a higher iodine value than that produced in the later part.

As regards the effects of gestation, Palmer and Isables (118) stated that gestation itself exerts no influence on the composition of either cow's milk or human's milk, but it may indirectly affect it by hastening the close of lactation which itself exerts marked changes on the composition of milk.

An interesting phase of this problem, which might have some bearing on the effect of lactation and gestation on the fat constants of butterfat, was studied by Maynard, Harrison and McGay (105). They recorded the changes in the total fatty acids, phospholipid fatty acids, and cholesterol content of the blood during the dry period and the early weeks of lactation when animals were fed according to produc-
tion and also at a constant level. Their data indicate a rapid rise in the lipid curves during the first part of the lactation and then a gradual decline as the production subsides, reaching a very low level during the dry period. Since the cows were fed according to production, the fat intakes were highest when the blood lipids were highest and vice versa. It might be concluded that the changes in blood lipids were merely the result of differences in intake and have no significant relationship to mammary activity. However, that same rise in the blood lipids following the initiation of milk secretion was observed in experiments with animals held at a constant level of food and fat intake during the dry period and the early weeks of lactation. This suggests that the stage of lactation has an influence upon the blood lipids independent of any effect which changes in the fat intake may exert.
Two experiments to determine the effect of cracked soybeans and linseed oil meal when each supplied the principal protein of the grain mixture fed to dairy cows upon the flavor of milk and cream are herein reported.

During the course of the first experiment, several important ways in which the experimental design and techniques might be improved were discovered. The second experiment was modified to incorporate these. Where necessary both experiments have been brought together for the purpose of clarifying the results.

A. Experiment I

1. Experimental Procedure

a. Animals used. Twenty Holstein cows of the Iowa State College herd were paired into two groups so that each pair was similar as to age, size, stage of lactation and production. Data concerning these cows are found in Table 1.

b. Feeding and management. The cows were fed alfalfa hay at the approximate rate of two pounds for each one hundred liveweight and grain at the rate of one pound for each three
pounds of milk produced. The grain mixtures were made up as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Mixture I Parts by wt.</th>
<th>Mixture II Parts by wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cracked corn</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>Oats</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Linseed meal</td>
<td>100</td>
<td>---</td>
</tr>
<tr>
<td>Cracked soybeans</td>
<td>---</td>
<td>100</td>
</tr>
<tr>
<td>Bonemeal</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Salt</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

All animals were fed thrice daily. The amounts of hay and grain eaten were weighed and recorded. Water was available to the cows at all times.

The cows were milked three times daily, at eight-hour intervals, and milk yields recorded. Only exercise given the cows was when they walked to the walk-through milking stalls, to the weighing scales, and to the judging pavilion when they were used in student judging classes. The butterfat test of each cow's milk was determined weekly from a one-day composite sample. Their health and condition were observed and recorded from day to day.
Table 1. Data on Cows Used in Experiment I

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Cow No.</th>
<th>Breed</th>
<th>Age at start of Experiment (Yrs.-Mos.)</th>
<th>No. of previous lactations</th>
<th>Days fresh at start of experiment</th>
<th>Liveweight at start of experiment (Lbs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1712</td>
<td>Holstein</td>
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<td>0</td>
<td>55</td>
<td>900</td>
</tr>
<tr>
<td>2</td>
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<td></td>
<td>2 - 1</td>
<td>0</td>
<td>48</td>
<td>900</td>
</tr>
<tr>
<td>1</td>
<td>1708</td>
<td></td>
<td>2 - 9</td>
<td>0</td>
<td>13</td>
<td>980</td>
</tr>
<tr>
<td>2</td>
<td>1696</td>
<td></td>
<td>2 - 5</td>
<td>0</td>
<td>13</td>
<td>980</td>
</tr>
<tr>
<td>1</td>
<td>1614</td>
<td></td>
<td>3 - 1</td>
<td>1</td>
<td>33</td>
<td>1120</td>
</tr>
<tr>
<td>2</td>
<td>1618</td>
<td></td>
<td>3 - 5</td>
<td>1</td>
<td>54</td>
<td>1071</td>
</tr>
<tr>
<td>1</td>
<td>1599</td>
<td></td>
<td>3 - 1</td>
<td>1</td>
<td>5</td>
<td>1227</td>
</tr>
<tr>
<td>2</td>
<td>1561</td>
<td></td>
<td>3 - 4</td>
<td>1</td>
<td>7</td>
<td>1226</td>
</tr>
<tr>
<td>1</td>
<td>1587</td>
<td></td>
<td>3 - 2</td>
<td>1</td>
<td>15</td>
<td>1113</td>
</tr>
<tr>
<td>2</td>
<td>1581</td>
<td></td>
<td>3 - 1</td>
<td>1</td>
<td>47</td>
<td>1195</td>
</tr>
<tr>
<td>1</td>
<td>1566</td>
<td></td>
<td>3 - 6</td>
<td>2</td>
<td>160</td>
<td>1284</td>
</tr>
<tr>
<td>2</td>
<td>1548</td>
<td></td>
<td>4 - 10</td>
<td>2</td>
<td>76</td>
<td>1210</td>
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<tr>
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<td>1557</td>
<td></td>
<td>3 - 6</td>
<td>1</td>
<td>46</td>
<td>1200</td>
</tr>
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<td></td>
<td>4 - 1</td>
<td>1</td>
<td>69</td>
<td>1305</td>
</tr>
<tr>
<td>1</td>
<td>1497</td>
<td></td>
<td>5 - 5</td>
<td>1</td>
<td>9</td>
<td>1152</td>
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<td>1505</td>
<td></td>
<td>3 - 8</td>
<td>1</td>
<td>32</td>
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</tr>
<tr>
<td>1</td>
<td>1544</td>
<td></td>
<td>5 - 5</td>
<td>3</td>
<td>60</td>
<td>1430</td>
</tr>
<tr>
<td>2</td>
<td>1584</td>
<td></td>
<td>4 - 9</td>
<td>2</td>
<td>34</td>
<td>1149</td>
</tr>
<tr>
<td>1</td>
<td>1523</td>
<td></td>
<td>7 - 6</td>
<td>3</td>
<td>48</td>
<td>1310</td>
</tr>
<tr>
<td>2</td>
<td>1539</td>
<td></td>
<td>7 - 9</td>
<td>3</td>
<td>75</td>
<td>1251</td>
</tr>
</tbody>
</table>
During the 70-day preliminary period of the experiment, both groups of cows received grain mixture I, which contained linseed meal as the principal source of protein. At the close of this period (January 6), the animals in group 1 continued to receive mixture I; while those in group 2 were changed to grain mixture II, which contained cracked soybeans as the principal source of protein. These rations were fed continuously for 74 days and then reversed (March 21). For the remainder of the trial, which was 49 days, group 1 received mixture II (cracked soybeans) and group 2 received mixture I (Linseed meal).

c. Collection and treatment of milk and cream samples.

(1) Milk samples. Individual milk samples were taken in glass bottles and scored for flavor approximately three times each week during the experiment. Composite milk samples were taken approximately twice each week during the last three months of the experiment. All sample bottles and experimental equipment were thoroughly cleaned and rinsed in chlorine water before being used. However, the samples were not always iced-down or protected from sunlight, which may account partly for the high percentage of observations of rancid and oxidized flavors obtained. Usually, the milk was scored within six hours after being drawn from the cows.
(2) Cream samples. At periodic intervals representative aliquot samples of a day's milk yield from each group of cows were separated promptly after each milking and the cream cooled to 40°F. as quickly as possible by means of an ice-bath. A clean separator was used for each batch of milk. The cream was held in a ten-gallon milk can. As soon as the 24-hour composite of cream was gathered, it was taken to the laboratory where it was churned, and the butter melted, centrifuged, and the fat filtered at a temperature of 60° - 70°C.

Some measure was needed to determine when the full effect of the feed on the milk fat was reached. After some consideration, the iodine number of the butterfat was selected as perhaps the best measure to use. It was supposed that the iodine numbers of the butterfat would soon stabilize themselves when the cows were fed a common ration. However, it became apparent as the experiment progressed that the iodine numbers did not stabilize as rapidly as had been supposed by some workers and that it was necessary to find out how long a time was necessary before making a "cross-over" of feeds. This period of time can be detected more easily and more accurately when the iodine number of butterfat is determined frequently, such as was done in this experiment. Many studies on the changes in fat composition reported by previous investigators have been made where the iodine number of butterfat was de-
58

determined only two or three times each month. This would obviously fail to reveal many of the fluctuations in iodine number and might tend to denote an early or late stabilization of the iodine number, either of which might be wrong. Since the literature did not disclose the trends in iodine numbers of butterfat except in relatively short-time feeding trials, a new objective was injected into the experiment: To determine the long-time effect of feed on the iodine numbers of butterfat. For this reason, there was no change in the feeding program following the one made at the end of the preliminary period until the latter part of the trial.

Iodine numbers of the milk fat were determined according to the Hanus method given by the A. O. A. C. (7) approximately every five days during a period of about six months. The butter oil was stored in glass sample jars at 10°C until analyses were completed. Ordinarily, this was accomplished within three-days' time.

2. Results and Discussion

a. Health and general condition of the cows. At no time during the experiment did the cows show a disinclination to eat their feed. One cow, 1548, was sent to the Veterinary Clinic for about ten days because of an abnormal swelling on the left anterior part of her udder. She was not used in
the experiment again until about two weeks after she returned to the dairy farm. Development of a severe case of mastitis made it necessary to drop her from the experiment about seven weeks before the termination of the trial. Coincidentally, the cow, 1556, which was paired with 1548 at the start of the experiment was also dropped from the experiment at the same time due to termination of her lactation. The data from these two cows were not used in the flavor studies. With the exception of minor disorders, which were considered without effect on the experimental results, the other cows were in excellent health for the duration of the experimental period.

Figure I shows that the mean gains in liveweights for groups 1 and 2 were 42 and 29 pounds respectively. For the most part, the conception data shown in Table 2 in conjunction with Figure I indicate that pregnancy did not influence the liveweights of the animals appreciably before the termination of the experiment.
Table 2: Conception Data for Cows Used in Experiment I

<table>
<thead>
<tr>
<th>Cow No.</th>
<th>No. of days during trial</th>
<th>Cow was open</th>
<th>Cow was with calf</th>
<th>Cow No.</th>
<th>No. of days during trial</th>
<th>Cow was open</th>
<th>Cow was with calf</th>
</tr>
</thead>
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<td>161</td>
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<td>42</td>
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<td>1528</td>
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<td>40</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1544</td>
<td>--</td>
<td>193</td>
<td>1518</td>
<td>90</td>
<td>103</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1556</td>
<td>--</td>
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<td>1539</td>
<td>--</td>
<td>193</td>
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<td>1548</td>
<td>193</td>
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<td>162</td>
<td>31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1712</td>
<td>123</td>
<td>70</td>
<td>1713</td>
<td>89</td>
<td>104</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. Curves Showing the Mean Liveweights of Cows Used in Experiment I.
b. Flavor criticisms of individual and composite milk samples. The effect of soybeans and linseed meal on the flavor of milk was studied. Certain cows in group 1, which received mixture 1 (Linseed meal), during the first of the experimental periods, consistently produced milk with a rancid flavor. When the milk from these cows was excluded from the composite milk sample, the flavor was improved considerably. On the other hand, the cows in group 2, which were fed mixture II (Cracked soybeans), produced milk quite free from rancidity. There was little change in the quality of the milk after the cows were switched to mixture I (Linseed meal). However, the incidence of rancid milk produced by the cows in group 1 diminished after they were fed cracked soybeans in the latter part of the experiment.

The data, shown in Table 3, indicate that factors other than feed were operating to cause the high incidence of rancidity in the milk of the cows in group 1 and that the individuality of the cow probably played an important role.

It is concluded from these data that the selection of cows to be used in studying the effect of feed on milk flavors should be based not only on such factors as breed, age, stage of lactation, etc., but also on the flavors of the milk they produce when fed the basal ration.
c. Relationship of certain factors to flavor criticisms of individual milk samples. Creamery operators have often suggested that one of the causes of off-flavored milk is the forcing of cows through heavy feeding to high milk and butter-fat yields. In this experiment, the cows were fed according to their production. If a cow showed an ability to respond to feed, then she was fed heavily to get the increased yield.

Some investigators of milk flavor (34, 80) have indicated that young cows were more apt to produce milk with a rancid or oxidized flavor than older ones. Data shown in Table 4 permitted a check on this factor. They show the total milk and fat yields of each cow during the experiment, the ages of the cows in days, and the percentage observations of off-flavor.

The differences in effect on milk flavor caused by mixtures I and II were minimized by the method of statistical analysis (145), between group correlations, as shown in Table 5. The conclusions drawn from this table will be discussed later on.

(1) Relationship between milk yield and fat yield, and percentage observations of feedy, oxidized, and rancid flavors. As shown in Table 5 the correlation coefficients between the total milk yield and fat yield, and percentage observations of feedy, oxidized, and rancid flavors were
Table 3. Flavor Scores and Criticisms of Composite Milk Samples from Each Group of Cows Used in Experiment I **

<table>
<thead>
<tr>
<th>Sampling Date</th>
<th>Group 1 Score</th>
<th>Criticism</th>
<th>Group 2 Score</th>
<th>Criticism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mixture I</td>
<td>Mixture II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jan. 30, 1941</td>
<td>20.0</td>
<td>Feedy &amp; Slightly Rancid</td>
<td>21.0</td>
<td>Feedy</td>
</tr>
<tr>
<td>&quot; 31</td>
<td>20.0</td>
<td>&quot; &quot; &quot; Rancid</td>
<td>21.0</td>
<td>&quot;</td>
</tr>
<tr>
<td>Feb. 3</td>
<td>20.0</td>
<td>Slightly Rancid</td>
<td>21.5</td>
<td>&quot;</td>
</tr>
<tr>
<td>&quot; 5</td>
<td>21.0</td>
<td>Feedy</td>
<td>19.5</td>
<td>Rancid</td>
</tr>
<tr>
<td>&quot; 8</td>
<td>21.0</td>
<td>Feedy</td>
<td>21.5</td>
<td>Feedy</td>
</tr>
<tr>
<td>&quot; 10</td>
<td>19.5</td>
<td>Rancid</td>
<td>21.5</td>
<td>Feedy</td>
</tr>
<tr>
<td>&quot; 13</td>
<td>19.0</td>
<td>Rancid</td>
<td>21.5</td>
<td>Feedy</td>
</tr>
<tr>
<td>&quot; 14</td>
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<td>Rancid</td>
<td>21.0</td>
<td>Feedy</td>
</tr>
<tr>
<td>&quot; 25</td>
<td>21.5</td>
<td>Flat &amp; Feedy</td>
<td>21.5</td>
<td>Flat &amp; Feedy</td>
</tr>
<tr>
<td>&quot; 26</td>
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<td>Flat &amp; Feedy</td>
<td>21.0</td>
<td>Flat &amp; Feedy</td>
</tr>
<tr>
<td>&quot; 28</td>
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<td>Feedy</td>
<td>21.0</td>
<td>Feedy</td>
</tr>
<tr>
<td>Mar. 3</td>
<td>18.0</td>
<td>Rancid</td>
<td>20.0</td>
<td>Feedy</td>
</tr>
<tr>
<td>&quot; 4</td>
<td>20.5</td>
<td>Slightly Rancid</td>
<td>21.5</td>
<td>Flat &amp; Feedy</td>
</tr>
<tr>
<td>&quot; 5</td>
<td>20.0</td>
<td>Feedy</td>
<td>21.5</td>
<td>Flat &amp; Feedy</td>
</tr>
<tr>
<td>&quot; 6</td>
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<td>Rancid</td>
<td>21.0</td>
<td>Flat &amp; Feedy</td>
</tr>
<tr>
<td>&quot; 10</td>
<td>21.0</td>
<td>Feedy</td>
<td>21.0</td>
<td>Feedy</td>
</tr>
<tr>
<td>&quot; 18</td>
<td>18.0</td>
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<td>21.0</td>
<td>Feedy</td>
</tr>
<tr>
<td>Mar. 21</td>
<td>19.5</td>
<td>Rancid &amp; Feedy</td>
<td>21.0</td>
<td>Flat &amp; Feedy</td>
</tr>
<tr>
<td>Apr. 10</td>
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<td>21.5</td>
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</tr>
<tr>
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<td>Feedy</td>
<td>21.5</td>
<td>Feedy</td>
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<td>21.0</td>
<td>Feedy</td>
</tr>
<tr>
<td>May 2</td>
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<td>Rancid</td>
<td>21.5</td>
<td>Feedy</td>
</tr>
<tr>
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<td>20.5</td>
<td>Feedy</td>
<td>21.0</td>
<td>Feedy</td>
</tr>
<tr>
<td>&quot; 9</td>
<td>21.0</td>
<td>Feedy</td>
<td>21.5</td>
<td>Feedy</td>
</tr>
</tbody>
</table>

* Milk from cows which gave rancid milk was excluded from the sampling.
** On March 21, Group 1 was changed from mixture I to II and Group 2 was changed from mixture II to I.
found to be non-significant. Thus, there is no evidence of a relationship between production and the occurrence of (1) feedy, (2) oxidized, and (3) rancid flavored milk in this experiment.

This work contradicts that reported by Henderson, Overcast, and Wylie (73). Their data indicate that a high level of feeding may tend to increase the susceptibility of milk to develop oxidized flavor. They reason that since milk production depends on the feeding level, cows in high production may produce milk more susceptible to oxidized flavor than during periods of lower production. However, Stobnitz and Sommer (147), and Hening and Dahlberg (33) found that the level of feeding has no effect upon the quality of the milk, the development of oxidized flavor in milk and the numerical score of aged milk.

MacCurdy and Trout (102) found that when cows were fed a given quantity of silage the feed flavor was more intense in milk from cows of least production. Slight feed flavors were noted in the milk when 0.79 pounds of corn silage or 0.40 pounds of alfalfa silage per pound of milk produced were fed to the cows one hour before milking. Feeding approximately 2.00 or more pounds of corn silage per pound of milk produced resulted in a strong feed flavor. Levels of feeding above a certain point did not seem to have a
<table>
<thead>
<tr>
<th>Cow No.</th>
<th>Age</th>
<th>Days</th>
<th>Milk</th>
<th>Fat</th>
<th>Off-flavor</th>
<th>Total</th>
<th>Feedy*</th>
<th>Feedy</th>
<th>Oxidized</th>
<th>Rancid</th>
</tr>
</thead>
<tbody>
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<td>8332</td>
<td>266</td>
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<td>72.2</td>
<td>36.5</td>
<td>36.5</td>
<td>3.25</td>
<td>46.16</td>
<td>15.39</td>
</tr>
<tr>
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<td>2800</td>
<td>6557</td>
<td>246</td>
<td>31</td>
<td>36.5</td>
<td>----</td>
<td>----</td>
<td>3.25</td>
<td>46.16</td>
<td>15.39</td>
</tr>
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<td>2052</td>
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<td>227</td>
<td>36</td>
<td>36.5</td>
<td>36.5</td>
<td>36.5</td>
<td>3.25</td>
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<td>31</td>
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<td>13.89</td>
<td>2.78</td>
<td>8.33</td>
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<td>6000</td>
<td>213</td>
<td>36</td>
<td>65.5</td>
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<td>13.89</td>
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<td>16.67</td>
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<td>14.71</td>
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<td>22.21</td>
<td>33.33</td>
<td>16.91</td>
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<tr>
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<td>7786</td>
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<td>36</td>
<td>66.6</td>
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<td>33.33</td>
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<td>2.50</td>
</tr>
<tr>
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<td>246</td>
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<td>8.33</td>
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<td>20.69</td>
<td>15.39</td>
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<td>5793</td>
<td>178</td>
<td>35</td>
<td>31.2</td>
<td>25.37</td>
<td>25.37</td>
<td>5.72</td>
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<td>8052</td>
<td>278</td>
<td>36</td>
<td>31.6</td>
<td>19.44</td>
<td>19.44</td>
<td>5.56</td>
<td>2.78</td>
<td>2.78</td>
</tr>
<tr>
<td>1581</td>
<td>1210</td>
<td>7966</td>
<td>254</td>
<td>35</td>
<td>57.2</td>
<td>3.68</td>
<td>3.68</td>
<td>31.45</td>
<td>11.42</td>
<td>6.00</td>
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<tr>
<td>1656</td>
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<td>6670</td>
<td>240</td>
<td>30</td>
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<td>30.00</td>
<td>30.00</td>
<td>30.00</td>
<td>10.00</td>
<td>10.00</td>
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<tr>
<td>1715</td>
<td>783</td>
<td>5522</td>
<td>211</td>
<td>30</td>
<td>33.4</td>
<td>26.87</td>
<td>26.87</td>
<td>60.00</td>
<td>6.57</td>
<td>6.57</td>
</tr>
</tbody>
</table>

* Includes those samples with "flat and feedy" criticism
Table 5. A Summary of the Statistical Treatment of Data Used to Study the Relationship of Milk Yield, Fat Yield, and Age to Flavor Criticisms of Individual Milk Samples from Cows Used in Experiment I

<table>
<thead>
<tr>
<th>Correlations Determined</th>
<th>$\bar{x}$</th>
<th>$Sx^2$</th>
<th>$\bar{y}$</th>
<th>$Sy^2$</th>
<th>$Sxy$</th>
<th>$\text{Correlation Coefficient}^2 (r)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A and D</td>
<td>7148</td>
<td>23,232,096</td>
<td>64.7</td>
<td>9151</td>
<td>-3,711</td>
<td>-0.00</td>
</tr>
<tr>
<td>A and E</td>
<td>7148</td>
<td>&quot;</td>
<td>15.2</td>
<td>5032</td>
<td>-36,526</td>
<td>-0.11</td>
</tr>
<tr>
<td>A and F</td>
<td>&quot;</td>
<td>&quot;</td>
<td>15.4</td>
<td>6488</td>
<td>-57,373</td>
<td>-0.17</td>
</tr>
<tr>
<td>B and D</td>
<td>245</td>
<td>19,122</td>
<td>64.7</td>
<td>9151</td>
<td>-1,148</td>
<td>-0.08</td>
</tr>
<tr>
<td>B and E</td>
<td>&quot;</td>
<td>&quot;</td>
<td>15.2</td>
<td>5032</td>
<td>-2,498</td>
<td>-0.26</td>
</tr>
<tr>
<td>B and F</td>
<td>&quot;</td>
<td>&quot;</td>
<td>15.4</td>
<td>6488</td>
<td>1,902</td>
<td>0.17</td>
</tr>
<tr>
<td>C and D</td>
<td>1466</td>
<td>6,257,912</td>
<td>64.7</td>
<td>9151</td>
<td>-30,334</td>
<td>-0.04</td>
</tr>
</tbody>
</table>

1. Letters A, B, and C refer respectively to (1) milk yield, (2) fat yield, and (3) age. Letters D, E, and F refer respectively to the percentage observations of (1) feedy, (2) oxidized, and (3) rancid flavors.

2. $Sxy / \sqrt{(Sx^2) (Sy^2)}$
further detrimental effect on the flavor. These data indicate that high producers, such as the ones used in this experiment, produce milk with less feed flavor than low producers. In this respect the two works are in agreement.

(2) Relationship between milk yield, fat yield, and age, and the percentage observations of flat flavor. As shown in Table 5a, the significant negative correlation coefficients of -0.61, -0.65, and -0.59 between flat flavor and (1) milk yield, (2) fat yield, and (3) age, respectively, indicate that as an animal increases in production and age there is less tendency for the production of milk having a flat flavor.

Usually flat flavor and the percentage of fat in the milk are associated together, the milk with the less butter-fat test having a "flatter" flavor. However, the writer wishes to emphasize that the total fat yields, and not fat percentages, were used in these comparisons.

(3) Relationship between age and the percentage observations of feedy flavor. The non-significant correlation coefficient (Table 5) between age and the percentage observations of feedy flavor indicates that there is no relationship between age and the occurrence of feedy flavor in milk.
(4) **Relationship between age and the percentage observations of rancid flavor.** The correlation coefficient between age and the occurrence of rancid flavor was 0.462 (Table 5a) which is about 0.004 from the significant point. One might be safe in saying that a relationship exists. Although the coefficient isn't significant the high value indicates that the tendency to produce rancid milk increases with the age of the cow.

(5) **Relationship between age and the percentage observations of oxidized flavor.** The correlation coefficient of -0.53 (Table 5a) found between age and the occurrence of oxidized flavor was significant, in fact almost highly significant. The results of Hening and Dahlberg (80), which were rather inconclusive, inferred that this relationship might exist.

Why young cows, especially first calf heifers, should have a greater tendency to produce milk more susceptible to the development of oxidized flavor than older cows is not known. Perhaps the explanation is that the younger animals, which produce milk susceptible to the development of oxidized flavor are forced from the herd leaving a much healthier group of older cows.

d. **Chemical constants of butterfat.** The iodine numbers, which were obtained weekly on samples of butterfat taken from
Table 5A. A Summary of the Statistical Treatment of Data Used to Study the Relationship
Fat Yield, and Age to Flavor Criticism of Individual Milk Samples from Cows

<table>
<thead>
<tr>
<th>Determined</th>
<th>( \bar{x} )</th>
<th>( Sx^2 )</th>
<th>( \bar{y} )</th>
<th>( Sy^2 )</th>
<th>( Sxy )</th>
<th>( r )</th>
<th>( b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A and G</td>
<td>7148</td>
<td>23,232,096</td>
<td>14.6</td>
<td>2102</td>
<td>-134,690</td>
<td>-0.61**</td>
<td>-0.0068</td>
</tr>
<tr>
<td>B and G</td>
<td>245</td>
<td>19,122</td>
<td>14.6</td>
<td>2102</td>
<td>-4,128</td>
<td>-0.66**</td>
<td>-0.22</td>
</tr>
<tr>
<td>C and E</td>
<td>1466</td>
<td>6,257,912</td>
<td>15.2</td>
<td>5032</td>
<td>-34,949</td>
<td>-0.55*</td>
<td>-0.052</td>
</tr>
<tr>
<td>C and F</td>
<td>&quot;</td>
<td>&quot;</td>
<td>15.4</td>
<td>6193</td>
<td>-93,055</td>
<td>-0.48</td>
<td>-0.049</td>
</tr>
<tr>
<td>C and G</td>
<td>&quot;</td>
<td>&quot;</td>
<td>14.3</td>
<td>2102</td>
<td>-69,322</td>
<td>-0.52*</td>
<td>-0.0095</td>
</tr>
</tbody>
</table>

1 Letters A, B, and C refer respectively to (1) milk yield, (2) fat yield, and (3) age.
2 Letters E, F, and G refer respectively to the percentage observations of (1) oxidized.

- Significant at the 5 per cent point
- Significant at the 1 per cent point
Statistical Treatment of Data Used to Study the Relationship of Milk Yield, Fat Flavor Criticisms of Individual Milk Samples from Cows Used in Experiment I

| $\bar{y}$ | $S_y^2$ | $S_{xy}$ | $(r)$ | $(b)$ | $S_{y|x}$ |
|----------|--------|---------|------|------|----------|
| 14.5     | 2102   | -134.380 | -0.31** | -0.0058 | 9.09     |
| 14.6     | 2102   | -41.128  | -0.65** | -0.22  | 3.69     |
| 15.2     | 5032   | -94.949  | -0.53*  | -0.0152 | 15.00    |
| 15.4     | 6433   | -95.056  | -0.462  | -0.0149 | 17.86    |
| 14.6     | 2102   | -63.322  | -0.52*  | -0.0095 | 2.423    |

The table above details the relationship between milk yield, fat yield, and age to the percentage observations of (1) oxidized, (2) renneted, and (3) flat flavors. The calculations involve

$$4. \sqrt{\frac{S_y^2 - S_{xy}^2}{S_x^2}} / (n-2) \quad 5. \frac{S_{y|x}}{\sqrt{S_x^2}} \quad \text{or} \quad b / S_b$$

These calculations are applied to each observed point.
the milk of cows on each of the experimental rations, are shown in figure 2. These curves indicate that the iodine numbers for both groups were practically the same during the preliminary period when they received mixture I (Linseed meal). Changes toward larger iodine numbers occurred in the milk fat produced by the cows in group 2 soon after they were placed on mixture II (Cracked soybeans). The full effect of the soybeans on the iodine number of the butterfat seemed to be attained in about 15 days after cows were changed to this ration. The differences in iodine numbers of the milk fats of the two groups were fairly constant until the rations were reversed (March 21).

Soon after the rations fed the two groups of cows were reversed the positions of the resultant iodine numbers were reversed. In approximately 15 days, a fairly constant difference in iodine numbers was established, which was smaller than during the previous period. This small difference was not maintained long for at slightly past the mid-point of the period, the curves approximately merged and remained so until the trial ended. This would indicate that factors other than feed were operating to influence the trend and magnitude of the iodine numbers. Of these factors changes in temperature may play an important role.
Figure 2. Curves Showing the Trends of the Iodine Numbers of the Butterfat from Cows Used in Experiment I.
Since the iodine number curves seemed to be lowest during the coldest months and tended to increase in magnitude toward spring it was thought that perhaps air temperature changes played a role in the fluctuations of the iodine numbers. As the barn temperature was not recorded, the mean external temperature, which was recorded at the Agronomy Farm (near the Dairy Farm), was used in this study.

Figure 3 indicates that the fluctuations of the iodine numbers and temperature are somewhat the same. As the temperature drops to a low level during the winter months the iodine numbers likewise decrease in value. As the temperature rises with the approach of spring the iodine numbers also increase. This relationship is illustrated differently in Figure 4, which shows the regression of iodine number on temperature changes.

Table 6 includes a summary of the statistical treatment of the data used to study the relationship between mean external temperature and the iodine numbers of the butterfat. The correlation coefficients between the temperature recorded and the iodine number indicate that temperature changes produce their greatest effect on butterfat composition 24 hours later. It is interesting to note, in this connection, that the correlation coefficients between the temperature on the same day, one day before, and two days before the samples were taken and iodine values are all highly significant.
Figure 3. Curves showing the relationship between the mean external temperature recorded one day before the butterfat samples were taken and the iodine numbers of butterfat from cows used in experiment I.
Figure 4. The Regression of the Iodine Number of the Butterfat from Cows Used in Experiment I on the Mean External Temperature Recorded One Day before the Cream Samples Were Taken.
Table 6. A Summary of the Statistical Treatment of Data Used to Study the Relationship
temperature and the Iodine Numbers of the Butterfat from Cows Used in Experimen.

<table>
<thead>
<tr>
<th>Correlations</th>
<th>X</th>
<th>Sx²</th>
<th>y</th>
<th>Sy²</th>
<th>Sxy</th>
<th>(r)</th>
<th>(b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>between Mean</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>External Temperature and Iodine Number</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A and Group 1</td>
<td>30.1</td>
<td>7473</td>
<td>33.14</td>
<td>124</td>
<td>696</td>
<td>0.72**</td>
<td>0.0931</td>
</tr>
<tr>
<td>B and Group 1</td>
<td>31.3</td>
<td>6405</td>
<td>33.17</td>
<td>133</td>
<td>755</td>
<td>0.82**</td>
<td>0.1178</td>
</tr>
<tr>
<td>C and Group 1</td>
<td>29.9</td>
<td>5888</td>
<td>33.17</td>
<td>133</td>
<td>661</td>
<td>0.74**</td>
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<td>A and Group 2</td>
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<td>33.94</td>
<td>51</td>
<td>317</td>
<td>0.61**</td>
<td>0.0424</td>
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<td>B and Group 2</td>
<td>31.3</td>
<td>6405</td>
<td>33.97</td>
<td>56</td>
<td>397</td>
<td>0.66*</td>
<td>0.0592</td>
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<tr>
<td>C and Group 2</td>
<td>29.9</td>
<td>5888</td>
<td>33.97</td>
<td>56</td>
<td>412</td>
<td>0.72*</td>
<td>0.0699</td>
</tr>
</tbody>
</table>

Letters A, B, and C refer, respectively, to the mean external temperatures recorded (1)
before, and (2) two days before the butterfat samples were taken.
Treatment of Data Used to Study the Relationship between Mean External
Temperatures of the Butterfat from Cows Used in Experiment I

<table>
<thead>
<tr>
<th></th>
<th>Sy²</th>
<th>Sxy</th>
<th>(r)</th>
<th>(b)</th>
<th>Sy.x</th>
<th>Sy.x</th>
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<tr>
<td>14</td>
<td>124</td>
<td>696</td>
<td>0.72**</td>
<td>0.0931</td>
<td>0.095lx+30.3377</td>
<td>1.2536</td>
<td>3.42**</td>
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<tr>
<td>17</td>
<td>133</td>
<td>755</td>
<td>0.32**</td>
<td>0.1173</td>
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<td>1.1024</td>
<td>3.30**</td>
</tr>
<tr>
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<td>651</td>
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<td>1.2975</td>
<td>5.54**</td>
</tr>
<tr>
<td>94</td>
<td>51</td>
<td>317</td>
<td>0.51**</td>
<td>0.0424</td>
<td>0.0424x+32.6653</td>
<td>0.9955</td>
<td>3.69**</td>
</tr>
<tr>
<td>97</td>
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<td>397</td>
<td>0.63*</td>
<td>0.0692</td>
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<td>0.9216</td>
<td>6.10*</td>
</tr>
<tr>
<td>97</td>
<td>56</td>
<td>412</td>
<td>0.72*</td>
<td>0.0699</td>
<td>0.0699x+31.8337</td>
<td>0.8876</td>
<td>6.10*</td>
</tr>
</tbody>
</table>

to the mean external temperatures recorded (1) the same day, (2) one day
before samples were taken.
Whether or not the correlation just indicated is wholly a function of temperature, is difficult to state. Advancement in lactation with pregnancy, as a result of changes in hormonal activity, may play a role in the changes on iodine number. It would appear, however, that temperature does have some effect on the magnitude of the iodine number, although its exact mechanism cannot be definitely postulated.

3. Summary and Conclusions

Twenty Holstein cows of the Station herd, divided into two equal groups, were used (1) to determine the time necessary for cracked soybeans to produce a maximum change in the flavor and quality of milk and butterfat, (2) to determine the variations in certain butterfat constants throughout a cow's lactation period and (3) to determine the feasibility of using the iodine number as a measure in ascertaining when a feed with a high oil content has exerted its maximum effect.

Individual milk samples were collected in glass bottles and scored at regular intervals throughout the experiment. Composite milk samples were collected during the last three months of the experiment. A determination was made of the iodine number of the butter oil from each group of cows.

The following points may be emphasized from the results of this experiment:
1. The selection of cows to be used in studying the effect of feed on milk flavor should be based not only on such factors as breed, age, stage of lactation, etc., but also on the flavors of the milk they produce when fed the basal ration.

2. When changes in fat composition are to be studied a control group of cows should be carried for each of the feeds being studied for the duration of the experiment.

3. Non-significant correlation coefficients were found between milk yield and fat yield, and the occurrence of feedy, oxidized, and rancid flavors in the milk.

4. Highly significant negative correlations of -0.61 and -0.65 between milk and fat yields, respectively, and the occurrence of flat flavor, indicate that as the total milk and fat production increases the tendency for the production of milk having a flat flavor decreases.

5. Likewise, a significant negative correlation of -0.52 between age and the occurrence of flat flavor indicates that as the age of the cow increases there is less tendency for the production of milk having a flat flavor.

6. Non-significant correlation coefficient was found between age and the occurrence of feedy flavor in the milk.

7. The correlation coefficient of 0.462, which is about .004 from the significant point, indicates that the
tendency to produce rancid milk increases with the age of the cow.

8. The significant negative correlation coefficient, -0.53, found between age and the occurrence of oxidized flavor indicates that the incidence of oxidized flavor is greater in the milk of young cows than in that of older cows.

9. In general, the maximum effect of a feed on fat composition, as measured by the iodine number, may be expected in approximately 15 days.

10. A direct relationship seems to exist between mean external temperature and the iodine number of the butterfat produced.

B. Experiment II

1. Experimental Procedure

a. Plan of experiment. In view of the results of the work conducted on this problem during 1941 the plan of this experiment was modified in two ways.

Certain features of the iodine number trends obtained in 1941 indicated the advisability of carrying two control groups of cows, one of which (group 3) was fed grain mixture I, containing linseed meal as the principal protein, and the other (group 4) was fed mixture II, containing cracked soy-
beans as the principal protein, continuously throughout the experiment. The other two groups (groups 1 and 2) were alternated from one ration to the other in succeeding periods throughout the experiment.

The data from 1941 also indicated that the effect of feed on the flavor of milk might be masked by the milk of certain cows which was constantly of an undesirable flavor. For this reason the experimental cows were selected on the basis of their milk flavor from a large group of cows that was being fed grain mixture I, containing linseed meal.

b. Animals used. The 12 Holstein and 4 Ayrshire cows which were selected on the basis of milk flavor during the preliminary period were divided into four similar lots based on breed, milk flavor, stage of lactation, milk and fat production, age, and size. By the method of randomization the lots were subdivided into four equal groups and the mixture which was to be fed each group determined. Data concerning these cows are found in Table 7. Animals having the same group number received the same feed.

c. Feeding and management. Alfalfa hay and grain were fed at the same rates as in the first experiment. Table 8 shows the composition of the concentrate mixtures.

Feeding, milking, and watering of the cows were handled as in the first experiment, except that the hay was not weighed. With a few exceptions the animals were weighed on
Table 7. Data on Cows Used in Experiment II

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>Number</th>
<th>Breed</th>
<th>Start of Experiment</th>
<th>Lactations</th>
<th>Start of Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>1614</td>
<td>Holstein</td>
<td>2 4 0</td>
<td>91 935</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1799</td>
<td>&quot;</td>
<td>2 5 0</td>
<td>111 1025</td>
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<td></td>
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<td>72 990</td>
</tr>
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<td>2 4 0</td>
<td>123 1103</td>
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<tr>
<td>II</td>
<td>1</td>
<td>1725</td>
<td>&quot;</td>
<td>3 2 1</td>
<td>68 1035</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1599</td>
<td>&quot;</td>
<td>4 2 2</td>
<td>40 1379</td>
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<tr>
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<td>1666</td>
<td>&quot;</td>
<td>3 10 1</td>
<td>76 1156</td>
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<td>4</td>
<td>1708</td>
<td>&quot;</td>
<td>3 4 1</td>
<td>32 1162</td>
</tr>
<tr>
<td>III</td>
<td>1</td>
<td>1692</td>
<td>&quot;</td>
<td>4 3 2</td>
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<tr>
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<td>2</td>
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<td>&quot;</td>
<td>4 2 2</td>
<td>19 1399</td>
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<td>1713</td>
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<td>3 3 1</td>
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<td>4 4 2</td>
<td>30 1172</td>
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<td>IV</td>
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<td>1630</td>
<td>Ayrshire</td>
<td>4 2 2</td>
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<td>2</td>
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<td>66 969</td>
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<tr>
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<td>&quot;</td>
<td>4 1 2</td>
<td>67 1022</td>
</tr>
<tr>
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<td>4</td>
<td>1625</td>
<td>&quot;</td>
<td>4 2 2</td>
<td>56 840</td>
</tr>
</tbody>
</table>

* 1810 was dropped from the experiment because of an injured teat and was replaced by 1427
Table 8. Ingredients of Grain Mixtures Used in Experiment II

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Mixture I Parts by weight</th>
<th>Mixture II Parts by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cracked corn</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>Oats</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Wheat bran</td>
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<td>300</td>
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<tr>
<td>Linseed meal</td>
<td>125</td>
<td>---</td>
</tr>
<tr>
<td>Cracked soybeans</td>
<td>---</td>
<td>125</td>
</tr>
<tr>
<td>Bonemeal</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Salt</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>
days that milk and cream samples were taken. All changes in health and condition of the animals during the experiment were recorded. The cows reacted negatively to the test for mastitis before the experiment began.

Butterfat tests were run on a composite sample of one day's milk collected once per week from each group of cows. The tests were made on the same day that samples of milk for chemical analysis were taken.

During the first experimental period, which was 39 days, Groups 1 and 2 were fed, respectively, the rations containing linseed meal (mixture I) and cracked soybeans (mixture II). Then the grain mixtures fed each of these animals were reversed and the alternate ration fed for a period of 74 days. At the end of this period the rations were again reversed and for 56 days the cows were fed the rations previously fed in period I.

Groups 3 and 4, which were designated as the control groups, were fed, respectively, the rations containing linseed meal and cracked soybeans for the duration of the experiment. The feeding schedules for the four groups of cows during the experiment are shown in Table 9.
Table 9. Feeding Schedules Followed in Experiment II

<table>
<thead>
<tr>
<th>Period</th>
<th>Reversal Groups</th>
<th>Control Groups</th>
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<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
</tr>
<tr>
<td>Preliminary</td>
<td>Mixture I</td>
<td>Mixture I</td>
</tr>
<tr>
<td>24 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>Mixture I</td>
<td>Mixture II</td>
</tr>
<tr>
<td>Experimental</td>
<td></td>
<td></td>
</tr>
<tr>
<td>39 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second</td>
<td>Mixture II</td>
<td>Mixture I</td>
</tr>
<tr>
<td>Experimental</td>
<td></td>
<td></td>
</tr>
<tr>
<td>74 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Third</td>
<td>Mixture I</td>
<td>Mixture II</td>
</tr>
<tr>
<td>Experimental</td>
<td></td>
<td></td>
</tr>
<tr>
<td>56 days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
d. Collection and treatment of milk and cream samples.

(1) Milk samples. All sample bottles and experimental equipment were thoroughly cleaned and rinsed in chlorine water before being used. Although milk samples from each cow were taken and scored for flavor as an aid in selecting and grouping the cows, this practice was discontinued at the beginning of the first experimental period. From then on composite samples were taken from a single milking of the cows which were being fed alike. These samples were taken in glass bottles about every five days. Great care was taken to see that the milk samples were always iced-down properly and stored in a special sample box, which protected them from light and provided a temperature of about 33°F. Usually, the milk was scored within six hours after being drawn. Twenty-four hours later these samples were scored again.

(2) Cream samples. Approximately one-eighth of the milk yield for one day from each group of cows was separated at 4-day intervals during the experiment. The cream obtained was placed in glass bottles, then stored and handled with the same care as were the milk samples.

Upon arrival at the laboratory the cream was heated to 143°F for 30 minutes to inactivate the lipases, and cooled immediately to 40°F. This was done to prevent the lipases
from producing a lipolytic action on the butterfat before the iodine and thiocyanogen numbers were determined. It was then scored for flavor and returned to the cooler (35°F). A second flavor score was obtained 24 hours later before the cream was discarded.

The cream, from which the fat for chemical analysis was obtained, was treated in the same manner as that in the first experiment. The milk fat was obtained by the same procedure as described previously. The iodine, thiocyanogen, and acid numbers of these fats were determined. In order to measure possible changes in the quality of the cream and milk fat, pH determinations of the cream and butter serum were obtained and acid numbers of the milk fat were determined.

e. Recording of barn temperatures. The high correlation found between mean external temperature and the iodine number of butterfat during the first experiment, prompted the keeping of a daily record of the barn temperature changes during the second trial.

Recording thermometers were installed in the two parts of the barn, where the cows were housed. During the winter months one part of the barn is usually a little colder than the other.

The experiment had been in progress about two months when one of the thermometers was broken. A replacement could
not be obtained. As only four animals were housed in this part of the barn it was felt that the experimental results would not be affected materially if the temperatures recorded by the other thermometer were used for all animals.

2. Results and Discussion

a. Health and general condition of the cows. Throughout the trial the cows remained in good health and condition, excepting cow No. 1810 which met with an accident about the middle of the trial. She injured the two rear quarters of her udder to such an extent that she had to be replaced. Cow No. 1427, similar in most respects, was substituted for her (March 27, 1942).

At no time during the experiment did the cows show a disinclination to eat their feed or a tendency to lose weight. The mean gains in liveweights were 35, 22, 55, and 36 pounds for groups 1, 3, and 4, respectively. Figure 5 shows a general upward trend in the weight curves as the experiment and lactation period progressed. This would be expected since well-fed cows usually increase in weight with the advancement of lactation. The rapid rise of the curve of group 3 beginning March 27 may be attributed to the substitution of the heavier animal, 1427, for 1810.
The conception data presented in Table 10 in conjunction with Figure 5 show that pregnancy did not influence the liveweights of the animals appreciably before the termination of the experiment. That the cows were in proper nutrition and healthy during the experimental period is indicated by the way in which they maintained their weight (Fig. 5) during the trials.
Figure 5. Curves Showing the Mean Liveweights of Cows Used in Experiment II.
b. A study of methods of obtaining cream samples for chemical analysis. The data shown in Tables 11 and 12 were obtained to determine whether a composite cream sample of one day's milking or a sample from one milking during the day should be taken for chemical analysis. Comparisons of the iodine and thiocyanogen numbers of the butterfat from each milking with those of the butterfat from a composite sample for the same day are presented in Table 11. Table 12 includes the pH determinations made on the cream and butter serum and the acid numbers of the butterfat from each milking and from a composite sample for the same day. The data presented indicate considerable variation among the iodine and thiocyanogen numbers and the acid values of the fat from milking to milking. In fact, there is as much variation between milkings as exists from day to day as determined by analysis of the composite samples. However, the average values for the three milkings approximate rather closely in most cases those obtained for the composite sample of the same day. Temperature fluctuations may be one of the factors responsible for the variations of the fat constants from milking to milking. With the exception of the data for January 23 differences among the pH values of the cream and butter serum (Table 12) from milking to milk-
Table 10. Conception Data for Cows Used in Experiment II

<table>
<thead>
<tr>
<th>Cow No.</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of days during trial</td>
<td>No. of days during trial</td>
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<td>No. of days during trial</td>
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<td>1725</td>
<td>23</td>
<td>169</td>
<td>1599</td>
<td>40</td>
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<td>1592</td>
<td>37</td>
<td>155</td>
<td>1504</td>
<td>26</td>
</tr>
<tr>
<td>1630</td>
<td>60</td>
<td>132</td>
<td>1528</td>
<td>44</td>
</tr>
<tr>
<td>1427</td>
<td>26</td>
<td>166</td>
<td>1806</td>
<td>26</td>
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<tr>
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<tr>
<td>1713</td>
<td>33</td>
<td>159</td>
<td>1613</td>
<td>134</td>
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<tr>
<td>1645</td>
<td>104</td>
<td>88</td>
<td>1625</td>
<td>101</td>
</tr>
</tbody>
</table>

* Replaced cow No. 1810 which was dropped from the experiment March 27
Table 11. Variations in the Iodine and Thiocyanogen Numbers and the Grams Iodine Equivalent to the Oleic Acid in 100 Grams Butterfat from Morning, Noon, and Evening Milks and from a Composite Milk Sample for the Same Day from Cows Used in Experiment II

<table>
<thead>
<tr>
<th>Date</th>
<th>Iodine Number</th>
<th>Thiocyanogen Number</th>
<th>Butterfat Oleic Acid</th>
<th>Grams Iodine per 100 Grams</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A.M. Noon P.M. Av. Comp.</td>
<td>A.M. Noon P.M. Av. Comp.</td>
<td>A.M. Noon P.M. Av. Comp.</td>
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</tr>
<tr>
<td>1-5-42</td>
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<td>32.03 32.92 31.40 32.13 32.11</td>
<td>29.71 30.75 29.32 29.92 29.67</td>
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</tr>
<tr>
<td>9</td>
<td>32.77 32.78 33.63 33.06 32.52</td>
<td>29.90 28.26 30.21 29.46 29.60</td>
<td>27.03 25.73 26.79 25.86 26.68</td>
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</tr>
<tr>
<td>12</td>
<td>32.90 32.69 34.05 33.21 33.24</td>
<td>30.13 30.02 31.42 30.52 30.17</td>
<td>27.36 27.35 28.79 27.83 27.10</td>
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</tr>
<tr>
<td>16</td>
<td>33.62 33.99 35.36 34.32 34.88</td>
<td>30.37 30.78 32.21 31.12 31.36</td>
<td>27.12 27.57 29.06 27.91 27.86</td>
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</tr>
<tr>
<td>19</td>
<td>31.64 31.89 32.00 31.91 31.33</td>
<td>29.08 29.51 28.87 29.15 29.40</td>
<td>25.32 27.13 25.74 26.39 26.87</td>
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</tr>
<tr>
<td>23</td>
<td>33.53 34.59 35.12 34.34 34.45</td>
<td>31.39 31.82 32.32 32.01 32.37</td>
<td>29.42 29.05 30.52 29.66 30.29</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>33.15 33.50 33.94 33.53 33.59</td>
<td>30.49 30.56 31.16 30.73 30.84</td>
<td>27.83 27.61 28.37 27.93 28.08</td>
<td></td>
</tr>
</tbody>
</table>
Table 12. Variations in the Acid Numbers of Butter Fat and the pH Values of Cream and Butter Serum from Morning, Noon, and Evening Milks and from a Composite Milk Sample for the Same Day from Cows Used in Experiment II

<table>
<thead>
<tr>
<th>Date</th>
<th>Samples:</th>
<th>pH of Cream</th>
<th>pH of Butter Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A.M. Noon P.M. Av. Comp.</td>
<td>A.M. Noon P.M. Av. Comp.</td>
<td>A.M. Noon P.M. Av. Comp.</td>
</tr>
<tr>
<td>9</td>
<td>.344 .831 .635 .603 .697</td>
<td>6.78 6.75 6.78 6.77 6.81</td>
<td>6.84 6.80 5.90 6.84 7.15</td>
</tr>
<tr>
<td>23</td>
<td>.340 .560 .609 .503 .620</td>
<td>7.29 7.07 7.08 7.04 6.85</td>
<td>7.00 6.76 6.90 6.35 6.86</td>
</tr>
<tr>
<td>Average</td>
<td>.365 .541 .507 .471 .468</td>
<td>6.88 6.84 6.81 6.84 6.82</td>
<td>6.98 6.94 6.96 6.96 6.94</td>
</tr>
</tbody>
</table>
ing are virtually within the experimental error of the method.

Thus, the variations encountered in the iodine and thiocyanogen numbers of the butterfat and the acid numbers of the butterfat from milking to milking indicated that daily composite cream samples should be taken.

A further study of the data presented in Table 11 shows that the iodine and thiocyanogen numbers of the butterfat from the evening milk are in most cases higher than those for the morning and noon milkings. The iodine equivalent to the oleic acid content of (Table 11) the butterfat (iodine number -(iodine number - thiocyanogen number) x 0.5) doesn't vary in this fashion as consistently as the iodine number.

As a check on the deterioration of the cream, from the time it was skimmed until churned, pH determinations on the cream and butter serum were made periodically. The pH values of the cream and butter serum approached the neutral point rather than showing a strong acid reaction. Likewise, the acid numbers of the butter oil, which were determined to indicate any abnormal lipase activity, were sufficiently low to assume that there was no appreciable lipase activity. These data, which are not shown, indicated that the cream samples did not change appreciably after collection and prior to the determination of the iodine and
thiocyanogen numbers.

c. Flavor scores and criticisms of raw milk and lipase-inactivated cream samples. Much difficulty was encountered in Experiment I in measuring the effect of the ration fed to the cows on the flavor of the milk they produced, because certain cows fairly consistently produced milk with a rancid or oxidized flavor. In some cases rancid flavor persisted after the cows were changed to a different ration, although its intensity varied from one ration to the other. This emphasized that factors other than feed were operating in causing certain flavors in the milk. These factors seemed effective in some cows and not in others.

It is highly probable that results of other studies on the effect of feed on milk flavors may have been distorted by factors other than feed without being recognized. Because of this the cows used in this experiment were all carefully selected after their individual milks were sampled and scored during a period of about four weeks. No cow that consistently showed abnormal flavors in her milk was used.

(1) Milk scores and criticisms. A study of Table 13 reveals that the scores and criticisms of the raw composite milk samples from each group of cows were practically the same. The milk from group 4 is the only one that received an oxidized flavor criticism when scored on the first day ("Oiliness" considered an oxidized flavor). The milk of
Collected in Glass Bottles from Each Group of Cows Used in Experiment II

<table>
<thead>
<tr>
<th>(2)</th>
<th>Group 3 (3)</th>
<th>Group 4 (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First Scoring</td>
<td>Second Scoring</td>
</tr>
<tr>
<td></td>
<td>Score</td>
<td>Criticism</td>
</tr>
<tr>
<td>35.0</td>
<td>F-Fi.</td>
<td>37.0</td>
</tr>
<tr>
<td>38.0</td>
<td>F</td>
<td>37.5</td>
</tr>
<tr>
<td>35.0</td>
<td>R</td>
<td>37.5</td>
</tr>
<tr>
<td>37.0</td>
<td>F</td>
<td>37.0</td>
</tr>
<tr>
<td>37.0</td>
<td>R</td>
<td>37.0</td>
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<tr>
<td>37.0</td>
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</tr>
<tr>
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</tr>
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</tr>
<tr>
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</tr>
<tr>
<td>37.0</td>
<td>F-Fi.</td>
<td>37.0</td>
</tr>
</tbody>
</table>

Flavor: 0 means Oxidized flavor, 1 means Oily flavor.
<table>
<thead>
<tr>
<th>Score</th>
<th>Criticism</th>
<th>Score</th>
<th>Criticism</th>
<th>Score</th>
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<th>Score</th>
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<tr>
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<td>R</td>
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<td>34.0</td>
<td>R</td>
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<td>F</td>
</tr>
<tr>
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<td>---</td>
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<td>36.5</td>
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<td>37.5</td>
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<td>37.5</td>
<td>36.4</td>
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</table>

ic flavor  O means Oxidized flavor  OL means Oily flavor

on April 7

ry 23 and from mixture II (Soybeans) to mixture I (Linseed) on April 7
group 1 received this criticism only once when scored the second day, while the milk of each of the other groups received this criticism twice. The criticisms for rancidity are also very few. This criticism did not occur for the milk of group 1, while the milk from each of the groups 2, 3, and 4 had one rancid criticism when scored on the first day. Samples from groups 1, 2, 3, and 4 were criticized as being rancid, respectively, (1) three, (2) five, (3) six, and (4) six times when scored the second time.

The first day average flavor scores were 37.4, 37.4, 37.5, and 37.3 for groups 1, 2, 3, and 4, respectively. The second-day scorings averaged 36.7, 36.1, 36.0 and 36.4 for groups 1, 2, 3, and 4, respectively. Differences between these scores are so small that their non-significance is obvious.

(2) Cream scores and criticisms. Table 14 shows that the cream scores and criticisms of the lipase-inactivated cream samples are almost identical with those of the milk. The samples of milk from groups 2 and 3 were criticized once as oxidized when scored on the first day. Samples from groups 1, 2, and 3 were each criticized twice as oxidized when scored the second time while those from group 4 criticized four times.

None of the samples of milk were criticized as rancid when scored on the first day, while group 2 was the only one to receive a rancid criticism at the second-day scoring.
Table 14. Flavor Scores and Criticisms of Lipase-inactivated Composite Cream Samples Col

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<thead>
<tr>
<th>Sampling Date</th>
<th>Group 1 (1)</th>
<th>Group 2 (2)</th>
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<td>&quot;  24</td>
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</table>

Average 37.9 37.6 37.92 37.4

Legend: F means Feedy flavor  F1 means Flat flavor  R means Rancid flavor

* Dates feeds were changed
1 Changed from mixture I (Linseed meal) to mixture II (Soybeans) on January 22 and from
2 "  "  "  II to "  "  "  I on " 23 "  "  3 Fed mixture I (Linseed meal) continuously
4 Fed mixture II (Soybeans) continuously
### Samples Collected in Glass Bottles from Each Group of Cows Used in Experiment II

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* Flavor 0 means Oxidized flavor, H means Heated flavor, O1 means Oily flavor

23 and from mixture II (Soybeans) to mixture I (Linseed meal) April 7
23 to II
The lesser occurrence of rancidity in the cream as compared with the milk may be attributed to inactivation of the lipases in the cream.

The average flavor scores on the first day are 37.9, 37.9, 37.8, and 37.9, for groups 1, 2, 3 and 4, respectively. Two-day scorings averaged 36.7, 36.1, 36.0, and 36.4 for groups 1, 2, 3, and 4 respectively. The differences between these scores are so small they are obviously non-significant.

It is concluded from these data that the effect of cracked soybeans and linseed meal on the flavor of raw milk and cream (heat-treated to inactivate lipases) are practically the same and not detrimental when either of these protein supplements constitutes 11.1 per cent of the concentrate fed to dairy cows.

d. Chemical constants of butterfat.

(1) Iodine number. Groups 1 and 2 were alternated from one ration to the other throughout the experiment, while groups 3 and 4 were fed mixture I (Linseed meal) and II (Cracked soybeans), respectively, for the duration of the experiment. The iodine and thiocyanogen numbers were employed as an index of the effect of feeds in changing butterfat composition.

(a) Effect of temperature. Results obtained in experiment I indicated that temperature changes play an
important role in the variations of the iodine number of butterfat. They pointed to a closer relationship between the iodine number of butterfat and the mean external temperature recorded one day before the samples were taken than the temperature recorded on the same day or two days before. This relationship between iodine number of butterfat and temperature was also evident in the results obtained in this experiment.

Table 15 shows that in general, highly significant correlations exist between the iodine numbers of the butterfat and the mean barn temperatures that were recorded the same day, one day before, and two days before the samples were taken. As the temperature rises the iodine values tend to increase and vice versa. This relationship is illustrated graphically in Figure 6. As in experiment I, a closer correlation exists between the temperature recorded one day before the samples were taken and the iodine number than that recorded on the same day or two days before. It is also interesting to note from Table 15 that a closer correlation exists between the temperature recorded the same day and the iodine number than that recorded two days before the samples were taken.
Table 15. Correlation Coefficients between Mean Barn Temperature and Certain Butterfat Constants of Butterfat from Cows Used in Experiment II

<table>
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<th>Correlation Coefficient between Temperature and</th>
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<th>Iodine No.</th>
<th>Thiocyanogen No.</th>
<th>Oleic Acid</th>
<th>Linoleic Acid</th>
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1. A, B, and C refer, respectively, to the mean barn temperatures recorded (1) the same day, (2) one day before, and (3) two days before the cream samples were taken.
2. Number of comparisons were 32
3. Grams Iodine calculated as equivalent to Oleic Acid in 100 grams butterfat
4. Grams Iodine calculated equivalent to Linoleic Acid in 100 grams butterfat
The high correlation of temperature changes with changes in the chemical composition of butterfat support the statement made previously in this manuscript that temperature fluctuations may be one of the factors responsible for the variations of fat constants from milking to milking and sample to sample even though the cows were fed the same feed.

(b) **Effect of feed.** Results obtained in the first experiment indicated that the maximum effect of feed on the changes in butterfat composition, as measured by the iodine number, may be reached in approximately 15 days after a change in feed. However, the data obtained in this experiment, which are shown in figure 6, indicate that the time necessary for the feeds to produce their maximum effect on butterfat composition is not clear, except after the first change of feeds. Comparisons of the iodine numbers of the butterfat from the reversal groups (groups 1 and 2) with those of the butterfat from the control groups (groups 3 and 4) have been made (Figure 6).

All animals were fed mixture I (Linseed meal) during the preliminary period (24 days). On December 15 groups 1 and 2 were changed, respectively, to mixtures I (Cracked soybeans) and II (Linseed meal); while group 3 continued
Figure 6. Curves showing the relationship between mean Barn Temperature Recorded One Day before the Samples Were Taken and the Iodine Number of Butterfat From Cows Used in Experiment II.
on mixture I and group 4 was changed to mixture II. It is noted from Figure 6 that after this change the differences between the iodine curves of groups 3 and 4 became greater than those between the curves of groups 1 and 2. These differences decrease in magnitude and seemed to stabilize themselves fairly well in approximately 20 days. Concurrent with this stabilization period was a sharp drop in temperature and likewise a downward trend of the iodine number curves of all groups. During the period December 15 to January 23 temperature and iodine number fluctuations were erratic.

On January 23 groups 1 and 2 were changed to mixtures II and I, respectively. A comparison of the iodine curves of these groups indicates that no definite effect resulting from change of ration was shown. There may be a change in the fat metabolism of the animals due to change of feed, for the curves of groups 1 and 2 cross each other twice soon after the change, whereas those of groups 3 and 4 do not.

From March 5 until April 7 the curves of (1) groups 1 and 2, and (2) groups 3 and 4 rise gradually and the curves of each pair approach each other more closely in magnitude. During part of this period, from March 5 until March 25, the variations in temperature were less than for
anytime during the experiment. Therefore, it seems logical to assume that the stage of lactation or of gestation may have played an important role in these changes in iodine number. At any rate, it is apparent that all groups were effected by the same factor or factors.

On April 7 groups 1 and 2 were changed to mixtures I (Linseed meal) and II (Cracked soybeans), respectively. Again, it is not possible to determine when the full effect of the feeds on butterfat composition was reached.

Figure 6 shows that the difference between the curves of groups 3 and 4 following the stabilization period (December 15) are small and that they are more consistent throughout the experiment than those between the curves of groups 1 and 2 which were alternated from one feed to the other. This might be interpreted as indicating that the cows adjusted themselves to the feeds fed in the quantities used in this experiment. As a result of such adjustment the iodine numbers (groups 3 and 4) have about the same magnitude regardless of the feed. The recurrent crossing of the curves of groups 1 and 2 might indicate that the fat metabolism of the cows was being disturbed by the changes in feed since the differences in iodine numbers were less constant than those of groups 3 and 4.
Several points should be emphasized from the data:

1. The maximum effect of feed on the changes in butterfat composition, as measured by the iodine and thiocyanogen numbers, appears to be reached in approximately 20 days after the first change of feeds (in the initial months of lactation) whereas the time necessary for them to produce their full effect following succeeding changes of feed is not indicated.  

2. When cows are fed either linseed meal or cracked soybeans as the chief protein supplement at no greater rates than were fed in this experiment over a long feeding period they seem to adjust themselves to the rations so that the iodine numbers of the butterfat are of about the same magnitude as those of a control group.  

3. On the other hand, when these feeds are fed for short feeding periods and then changed, the fat metabolism of the cows seems to be disturbed so that the differences between the iodine numbers of the butterfat are not very consistent. However, over a period of time the variations in iodine numbers caused by the change from one feed to the other may be balanced out, such as occurred in this experiment with groups 1 and 2 (averages for the entire experiment were 35.10 and 35.34 for groups 1 and 2, respectively.
(2) Thiocyanogen number. Figure 7 shows that the trends of the thiocyanogen curves and their relationship to temperature changes are similar in most respects to those of the iodine number curves. Generally, what was said about iodine numbers and the factors that influence them also applies to the thiocyanogen numbers. This is expected for the following reasons: (1) In compounds having single ethylenic linkages, as oleic acid, thiocyanogen adds quantitatively as do the usual halogenation reagents. (2) When two unsaturated linkages occur as in linoleic acid the thiocyanogen adds to but one of these, whereas with normal iodination methods halogen adds to both linkages. (3) Oleic acid is the chief unsaturated fatty acid of butterfat. Since both iodine and thiocyanogen add to the single ethylenic linkage of oleic acid, variations in its content (such as were obtained in this experiment and which will be discussed later) would naturally reflect similar changes in the iodine and thiocyanogen numbers.

(3) Grams iodine per 100 grams butterfat equivalent to oleic acid and linoleic acid. Comparisons among figures 8, 7 and 8 show that the trends of the iodine, thiocyanogen, and oleic acid (expressed as grams iodine per 100 grams butterfat equivalent to oleic acid) curves
Figure 7. Curves showing the relationship between barn temperature recorded one day before the samples were taken and the thiocyanogen number of butterfat from cows used in Experiment II.
are almost identical for each group. This is particularly true of the thiocyanogen curves of groups 3 and 4 (controls) shown in Figure 7. Since iodine and thiocyanogen adds quantitatively to the single ethylenic linkage of oleic acid and since there is more oleic acid in butterfat than any of the other unsaturated fatty acid one would expect variations in the oleic acid content of butterfat to result in similar variations of the iodine and thiocyanogen numbers.

A significant correlation coefficient was found between the temperature recorded one day before the samples were taken and the oleic acid content of the butterfat (Table 15). With two exceptions the relationship between temperature and linoleic acid (expressed as grams iodine absorbed by linoleic acid per 100 grams butterfat) was non-significant. The data presented in Figure 8 and Table 15 indicate that the changes in iodine number were largely dependent on the changes in the oleic acid content of the butterfat.

e. The relative effect of linseed meal and cracked soybeans on the hardness of butterfat as measured by the iodine number. Although many factors, such as moisture content, churning and working conditions, etc. of the butter may influence its hardness, Coulter and Hill (36) found that the hardness of butter, made under uniform
Figure 8. Curves Showing the Relationship between Mean Barn Temperature Recorded One Day before the Samples Were Taken and the Grams Iodine Per 100 Grams Butterfat Equivalent to Oleic Acid of Butterfat from Cows Used in Experiment II.
conditions, is closely dependent upon the hardness of the butterfat. Likewise, the Minnesota workers (36) found a highly significant correlation between the hardness of butterfat and the iodine number of the butterfat. They point out that extreme variations in the Reichert-Heissl number of the butterfat may be associated with variations in the hardness of the butterfat.

A study of the data shown in Figure 6 shows that the iodine numbers of groups 3 (fed linseed meal) are generally higher than those of group 4 (fed cracked soybeans). Moreover, an analysis of variance reveals that these differences, which are one unit or less in most cases, are significant (F=4.19*). However, it is doubtful whether a difference of one unit in iodine value would greatly affect the commercial processing of butter. Analysis of variance of the iodine numbers of the butterfat from groups 1 and 2 revealed no significant difference between them. Their averages (35.10 and 35.34 for groups 1 and 2, respectively) for the entire experiment and the recurrent crossing of their curves indicate that the differences between their iodine numbers were balanced out during the experiment. However, the writer wishes to point out that although the differences in the iodine numbers of the butterfat from groups 1 and 2 were balanced out during the experiment, the differences were
great enough at certain periods to cause different effects on the quality of butter. For example, the differences in the iodine numbers of groups 1 and 2 from April 7 to April 15 and from April 25 to June 1 would indicate that one could expect a difference in the body of butter produced by cows fed cracked soybeans or linseed meal at the same rates as were fed in this experiment. The data shown in Figure 6 indicate that while the iodine numbers of the butterfat from cows fed either of these two feeds may be practically the same (especially over a long feeding period) at certain times, they may also be quite different at different temperature levels or at different stages of lactation or gestation. The feeding program used for the cows of groups 1 and 2 would not be normally used by a dairy farmer, whereas that used for groups 3 and 4 would be more generally accepted.
3. Summary and Conclusions

Sixteen cows from the station herd were divided into four similar lots, based on breed, milk flavor, stage of lactation, milk and fat production, age, and size. By the method of randomization the lots were subdivided into four equal groups which were fed predetermined ration, similar except as to the protein supplement. In one case the supplement was linseed meal and in the other cracked soybeans. Groups 1 and 2 were fed these two rations by the double reversal method while group 3 was fed the ration containing cracked soybeans and group 4 the one containing linseed meal continuously throughout the experiment.

Composite milk and cream samples were collected in glass bottles at regular intervals throughout the experiment and scored. The cream was heated sufficiently to inactivate the lipase before scoring, while the milk was scored in the raw state. Usually the samples were scored again 24 hours later before being discarded.

The iodine and thiocyanogen numbers were determined from composite samples of milk fat collected from the cows in each group. Acid numbers of the butterfat and pH determinations of the cream and butter serum were made to determine whether or not bacterial activity would indicate reduction in the keeping quality of the cream and conse-
sequently the milk fat, and to determine whether the samples had received proper handling prior to analysis.

Results of the first experiment indicated the advisability of recording the barn temperature daily during the trial. Recording thermometers were installed in the barn for this purpose.

The following conclusions are drawn from this experiment:

1. To determine changes in fat composition, it seems advisable to utilize composite samples of one day's milk yield rather than samples from individual milkings.

2. Highly significant correlations exist between the barn temperatures (recorded the same day, one day before, and two days before the samples were taken) and the iodine and thiocyanogen numbers of butterfat. These results support the findings of experiment I in that air temperature affects the iodine number of butterfat.

3. The mean barn temperature recorded one day before the samples were taken has a closer correlation to changes in fat composition than that recorded the same day or two days before.

4. The maximum effect of feed on the changes in butterfat composition, as measured by the iodine and thiocyanogen numbers, appears to be reached in approximately 20 days.
after the first change of feeds (in the initial stages of lactation) whereas the time necessary for them to produce their full effect following succeeding changes of feed is not indicated by the data obtained.

5. When cows are fed either linseed meal or cracked soybeans as 11.1 per cent of the concentrate mixture over a long feeding period they appear to adjust themselves to the rations so that the iodine numbers of their butterfat are of about the same magnitude and their differences fairly consistent.

6. On the other hand, when these feeds are fed for short feeding periods and then changed the fat metabolism of the cows seems to be disturbed so that the differences between the iodine numbers of the butterfat are not very consistent.

7. The changes in iodine number were largely dependent on the changes in the oleic acid content of the butterfat.

8. Non-significant differences were found between the iodine numbers of the butterfat from cows alternated from the linseed meal ration to the soybean ration and vice versa.

9. A significant difference was found between the iodine numbers of the butterfat from cows fed linseed meal and cracked soybeans at the rate of 11.1 per cent of the concentrate mixture. The iodine numbers of the butterfat from
cows fed the linseed meal were generally higher throughout the experiment than those of the butterfat from cows fed cracked soybeans.

4. A Discussion of Certain Aspects of the Results of Experiments I and II.

A comparison of certain data obtained in the first experiment with those obtained in the second experiment reveals that they are similar in some respects and different in other respects.

The results of the first experiment (Figure 2) indicate that the maximum effect of a feed on the changes in butterfat composition, as measured by the iodine number, may be expected in approximately 15 days after a change of feeds (both Jan. 7 and March 21). Data obtained in the second experiment (Figure 5) indicate that the full effect may be reached in approximately 20 days after the first change of feed (December 15 in the initial stages of lactation), whereas the time required for it to produce its full effect following succeeding changes is not indicated. A comparison of the data obtained in corresponding experimental periods of both experiments may partially explain these differences.

The data obtained in the first experimental period (January 7 to March 21) of experiment I corresponds fairly
well with those obtained in the same period (December 15 to January 23) of experiment II with respect to the time necessary for the feeds to produce their maximum effect on the changes in butterfat composition. It is noted that the experimental period of the first experiment was much longer than the one of the second experiment. The second (last) experimental period (March 21 to May 5) of experiment I and the corresponding one (January 23 to April 7) of experiment II are similar in that the iodine numbers of the butterfat from the cows do not maintain the fairly consistent differences after the second feed change which were characteristic of them after the first feed change. Although it does seem that the differences between the iodine numbers of the butterfat from the cows fed mixtures I and II in the first experiment become fairly consistent in approximately 15 days after the change of feeds, this consistency is maintained for only a short time after the second feed change. In this case the second experimental period of experiment I was much shorter than that of the second experiment and as regards season and lactation period corresponds more nearly to the third period of experiment II. One common characteristic of the iodine numbers of both experi-
ments during April and May is that they were more erratic with less consistent differences than were obtained during the first experimental period.

Thus it appears from these comparisons that when cows are fed either linseed meal or cracked soybeans as 11.1 per cent of the concentrate mixture over a long feeding period they may adjust themselves to the rations (especially when the feeds are started early in the lactation period) so that the iodine numbers of their butterfat are of about the same magnitude and their differences fairly consistent. On the other hand, when these feeds are fed for short feeding periods and then changed the fat metabolism of the cows may be distributed so that the differences between the iodine numbers of the butterfat are not very consistent.

In experiment I, the iodine numbers of the butterfat from cows fed the soybean ration were generally higher throughout the experiment than those of the butterfat from cows fed the linseed ration. They were the reverse in the second experiment and had less differences between them. These differences may be due to variations in the oil content of the two feeds, which may be affected by numerous factors, such as soil and climatic conditions, processing, etc. It would be wise in future studies of changes in fat composition to make a periodic analysis of the feeds being studied.
The relationship between air temperature and the iodine number of butterfat was studied in both experiments. The external temperature was used in the first experiment, while barn temperature was used in the second one. Results obtained in the first and second experiments (Figures 3 and 6) indicate that air temperature affects the iodine number of butterfat. In both experiments a closer correlation was found between the temperature recorded one day before the samples were taken and the iodine number of the butterfat than that recorded the same day or two days before. Also, the temperature recorded the same day had a closer relationship to the iodine number of the butterfat than that recorded two days before.

In view of the close relationship found between temperature and the iodine number of butterfat it seems logical that the high iodine numbers obtained in April and May may be partially a function of changes in fat metabolism of the cow due to the rise in temperature rather than the effect of feed or stage of lactation and gestation which has been suggested by some investigators.
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