A STUDY OF THE MECHANISM BY WHICH HOMOGENIZATION INFLUENCES FLAVOR DEVELOPMENT IN MILK

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INTRODUCTION

Flavor defects due to chemical reactions are often encountered in high quality market milk as it reaches the consumer. One of the most prevalent and serious flavor defects in the market milk industry today is the one known as "oxidized". This flavor is also known as tallowy, metallic, cardboard, cappy, papery, oily and emery, dependent largely upon its intensity. There are numerous causes and factors involved in the development of oxidized flavors in milk, which have resulted in many complications in regards to its control.

Contamination of milk with copper and iron has long been known to catalyze oxidized flavor development. Within recent years, extensive investigations have been conducted showing the effects of feed, carotene, vitamin C (ascorbic acid), lecithin, enzymes, bacterial action and apparent acidity on oxidized flavor development. More recently, the effect of heat treatment upon the development of oxidized flavor has received considerable attention.

Recent research has shown that the homogenization of milk at sufficiently high pressures prevents or retards the development of oxidized flavor, even when the milk is highly contaminated with such catalytic metals as copper and iron. However, no one has shown by what means homogenization protects milk against such flavor development. Whether the mechanism, by which the milk is stabilized, is chemical, physical, or a combination of both remains to be shown. Likewise, the exact cause of hydrolytic rancidity in homogenized raw milk is undetermined and needs further study. The object of this study is to determine, if possible, how the homogenized milk is stabilized against oxidized flavor development and what relation it has, if any, to hydrolytic rancidity in homogenized raw milk.

REVIEW OF LITERATURE

I. Oxidized Flavor of Milk

Introduction. Since Golding and Feilmann (1905) first called attention to a developed "alkaline mealy flavor" which was "driving away the custom of the retailer", a flavor resulting from copper contamination, many data have been collected on the oxidized flavor of milk. Considerable of these data are without the scope of this paper, but the factors responsible for the development of oxidized flavor may be so closely related to the retarding effect of homogenization on oxidized flavor development that inclusion herewith of the salient facts seems desirable.

Frequency of oxidized flavor. Roland and associates (1937) studied the frequency of oxidized flavor defects in commercial market milk from 19 dairies and classified 20.9 per cent of the samples as being oxidized to some degree, with the defect being the most troublesome in the high-fat, premium quality milk. Hening and Dahlberg (1939) found the occurrence of oxidized flavor in pasteurized milk at 24 hours after pasteurization, reaching a high in March, being absent from May to September inclusively, and recurring in October. The milk scored on the third day after pasteurization showed somewhat the same general trends as that scored the first day after pasteurization, excepting that the frequency of oxidized flavor was much greater. Roadhouse and Henderson (1935) reported that 24.2 per cent of 349 samples showed oxidized flavor. Among the pasteurized samples 35.3 per cent were oxidized; among the raw samples only 18.2 per cent showed the defect.

Roland and associates (1937) found the percentage occurrence of oxidized flavor in raw milk started down in April, went up in May, was zero in August and September and increased again in October and November. Brown and associates (1937a) stated that mixed raw milk never developed oxidized flavor when kept free from iron and copper contamination. Dahle and Palmer (1937) reported that a temperature of 145° F. for 30 minutes greatly accentuated the off-flavor. Greenbank (1936) pointed out that oxidized flavor developed more rapidly when stored at 5° C. than at 15° C. Trout (1937) noted that 5.5 per cent of the samples of pasteurized milk studied over a six weeks period in late summer showed oxidized flavor development the first day after pasteurization with an increase up to 20.7 per cent on the third day.

<u>The effect of season on oxidized flavor.</u> The greater occurrence of oxidized flavor in winter milk has resulted in much investigation along this line. Guthrie and Brueckner (1933) found that the oxidized flavors were more pronounced and more widespread in winter than in summer. Anderson and associates (1937) stated that a sudden hot humid weather caused cows which had been producing off-flavor milk to produce good milk temporarily, while sudden cold spells appeared to aggravate off-flavors making them more intense. Mattick (1927) believed the contributing factors were probably limited by a rise in temperature which corresponded to summer conditions.

Webb and Hileman (1937) have shown that summer milk is able to resist the development of oxidized flavor even in the presence of high oxidation-reduction potentials.

Hening and Dahlberg (1939) found the frequency of oxidized flavor in pasteurized milk to be high in March, to be absent from May to September and to recur in October.

Garrett and Bender (1940) observed that milk produced during the summer by simulated winter feeding conditions was more susceptible to the development of oxidized flavor than was milk produced by typical pasture feeding, and concluded that season seemed only incidental and the type of roughage was the important factor.

<u>The cow as a source of oxidized flavor.</u> Many investigators have studied the cow as a source of oxidized flavor. Guthrie and Brueckner (1933) found that 21 per cent of 155 cows studied gave milk consistently in which distinct oxidized flavors developed after pasteurization on three days storage, while another 10 per cent produced milk in which oxidized flavors developed only slightly. Dahle and Palmer (1937) found that approximately 37 per cent of the cows yielded milk during March which developed an oxidized flavor. This dropped to 25 per cent and 5 per cent respectively in May and October.

Both Guthrie and Brueckner (1933) and Dahle and Palmer (1937) stated that apparently no relation existed between the breed or stage of lactation and the development of the oxidized flavor in the milk. Hening and Dahlberg (1939) found that milk from first calf heifers showed a higher incidence of oxidized flavors than milk from older cows, while Guthrie and Brueckner (1933) reported there was no relation between the age of the cow and the development of the oxidized flavor.

Beck, Whitnah and Martin (1939) reported that oxidized flavor occurred in 6.1 and 7.8 per cent respectively of 480 samples of Jersey and Guernsey milk and in 15.8 and 19.4 per cent respectively of 448 samples of Holstein and Ayrshire milk.

Greenbank (1936) found that one cow which had just freshened produced milk which throughout her lactation showed oxidized flavor on storage and concluded that this defect may not necessarily always develop late in the lactation period.

Brown and associates (1937a), Fox (1937) and Tracy and associates (1933) reported that there was considerable variation among individual cows with respect to the tendency for oxidized flavor to develop in the milk. The latter investigators thought this difference to be due to cells or other anti-oxidizing substances contained in milk. Stebnitz and Sommer (1937b) stated there were considerable variations in the stability of the butterfat toward oxidation from different cows and from an individual cow at different times.

Guthrie and Brueckner (1933) observed that oxidized flavors were found in foremilks, middle milks and last milks; being a little more noticeable in the foremilks, which may have been due to a smaller amount of butterfat in the milk. There was a variation in the intensity of the oxidized flavor which developed in the milk from different quarters of the udder, indicating that dry feeds were not the sole cause of the development of the flavor. The oxidized flavor appeared in the milk of some cows for several weeks at a time; the milk from others was irratic in this respect.

Relationship of feed to oxidized flavor. Brown and associates (1937a) found that changing cows from dry feeding to dry feeding plus pasture caused the milk to become non-susceptible to oxidized flavor development. Apparently pasture grasses contain one or more substances which pass into the milk where they act in such a manner as to retard or prevent oxidized flavor development even though copper or iron is added.

Stebnitz and Sommer (1937b) found that fat from cows receiving grass in their ration was less saturated and hence more susceptible to oxidation, but the presence of increased amounts of protecting substances in pasture produced milk prevented the development of the defect. Thurston (1935), Dahle and Palmer (1937) and Greenbank (1936) have reported the ability of grass pasture to stabilize milk flavor. Greenbank (1938b) noted that the inhibition of oxidized flavor when cows were fed green feed was paralleled by a decrease in oxidation-reduction potential and an increase in poising action.

Garrett and Bender (1940) found that milk produced during the summer by simulated winter-feeding conditions was more susceptible to the development of oxidized flavors than was milk produced by pasture feeding.

Brown and associates (1937a) stated that so-called industrial feeds, especially dried beet pulp lacked reducing substances thus seeming to favor oxidized flavor development.

Hening and Dahlberg (1938b) found that the feeding of mangels apparently exerted no influence in preventing the development of oxidized flavor. Dried beet pulp neither prevented nor increased the susceptibility of the milk toward oxidized flavor defects. In an earlier work (1938a) they found that the level of feeding did not have any effect on the flavor of milk or the frequency of oxidized flavor.

Prewitt and Parfitt (1935) showed that the ration containing soybean oil either direct or in the unprocessed beans had a tendency to produce milk which upon holding developed less degree of oxidized flavor than did milk from cows fed other rations.

Fox (1937) found little relationship between various rations and oxidized flavor development. However, some milks had a lesser tendency to become oxidized when the cows were placed on a low fat ration.

Liebscher (1937) observed no effect on the taste or copper content of milk when cows were fed beet tops treated with copper.

<u>The effect of apparent acidity on oxidized flavor.</u> Anderson, Dowd and Steuwer (1937) found that high acid milk of about 0.19 per cent acidity frequently developed oxidized flavor after 48 hours storage. When these samples were neutralized until the acidity was 0.15 or below, none of them developed an oxidized flavor in 48 hours. The degree of acidity at which the development of oxidized flavor was retarded or prevented varied with different milks from different sources. They explained that the retarding effect of neutralization on the development of oxidized flavor was due to a balancing of the sodium-calcium-phosphorus-casein complex in milk.

However, Brown and Dustman (1939) could find no relation between the acidity of freshly drawn milk and the tendency to develop oxidized flavor. Both normal and oxidized flavored milk were found in high and in low acid milks in about the same proportions. The standardization of the acidity of milk to 0.13 per cent did not affect the tendency for the milk to develop oxidized flavor when contaminated with copper.

Greenbank (1936) increased the pH of milk 0.1 and found it sufficient to prevent the development of oxidized flavor after 24 hours storage and decreased the intensity after 48 hours storage.

<u>Effect of bacterial action on oxidized flavor.</u> Since some bacteria are known to have reducing powers it would seem probable that they would lower the tendency for milk to become oxidized. Mattick (1927) suggested that bacteria, although not directly involved in the reaction, act in a retarding capacity by their own utilization of oxygen, or by the production of acidity which carried the system outside the limiting pH.

Tracy et al (1933) working with living yeast cells found that they retarded the development of tallowiness in milk stored at 40° F. Dead

cells or the filtrate of a yeast suspension had no such effect. The bacteria and yeast caused a change in potential towards the reduction phase, suggesting that a removal of oxygen occurred through the metabolism process of the organisms.

Roland and associates (1937) found there was a marked tendency for samples falling in the oxidized flavor group to show considerable lower bacterial counts than those free from oxidized flavor.

Dahle and Palmer (1937) found there was no significance between particular bacterial counts and oxidized flavor, although high counts retarded the development of the defect. Incubation also helped retard the development of oxidized flavors. Both incubated and unincubated pasteurized samples developed a very strong flavor. They suggested that the heat treatment in addition to the destruction of bacteria affected some reducing substance which incubation could not restore. Dahle (1938) found that starter bacteria would delay the development of the off-flavor. Tracy and associates (1933) found that incubation retarded the development of tallowy flavors.

Relation of ascorbic acid to oxidized flavor.

a. Disappearance of ascorbic acid and oxidized flavor development. Chilson (1935) reported that all the ascorbic acid in milk was found to have been oxidized by the time oxidized flavor was detectable by taste. He pointed out that the oxidizing enzyme in the skimmilk was the major factor involved in causing the destruction of ascorbic acid when the milk was pasteurized at 143° F. without the presence of metals.

Dahle and Palmer (1937), Sharp and associates (1936), Trout and Gjessing (1939) and Guthrie and associates (1939) have confirmed the work of Chilson's and reported a close correlation between the disappearance of ascorbic acid and the development of oxidized flavor.

Sharp et al (1936) stated that the accelerating effect of soluble copper on the development of the oxidized flavor and the oxidation of ascorbic acid was more pronounced in the presence of the active enzyme.

Trout and Gjessing (1939) found the rate of disappearance of ascorbic acid was greater in winter milk than in summer milk, being relatively greater in the irradiated and in the grade A than in the pasteurized milk, and when a distinct oxidized flavor was noted, little or no ascorbic acid was present.

Guthrie and associates (1939) stated that the relationship between the destruction of ascorbic acid and the development of oxidized flavor was often so definite that conditions affecting the development of the oxidized flavor could be followed indirectly by studying the ascorbic acid and factors influencing its disappearance. Henderson (1939) found no direct connection between the complete destruction of ascorbic acid and the development of oxidized flavor.

b. Effect of light, oxygen and metals on ascorbic acid. The destructive action of metals, especially copper, on ascorbic acid has been observed by Chilson (1935), Guthrie et al (1939) and Josephson and Doan (1939).

The presence of oxygen is thought to accelerate the oxidation of ascorbic acid. Dahle and Palmer (1937) found the ascorbic acid content of milk to diminish in the absence of oxygen but not so rapidly as in samples containing free oxygen. However, Guthrie and associates (1939) obtained results showing that even in the presence of sunlight the oxidation of ascorbic acid did not occur in oxygen-free milk. Hand et al (1938) heated milk at 63° C. for three hours after the addition of 0.1 milligram per liter of dissolved copper and found no appreciable oxidation of reduced ascorbic acid. When copper treated milk was vacuum cooled no destruction of ascorbic acid was noted.

Light has been found to have a very destructive action on the ascorbic acid in milk. Hand and associates (1938) believed lactoflavin to be the sole agent in milk responsible for the sensitivity of ascorbic acid to light. Guthrie et al (1939) reported that the ascorbic acid might disappear within five minutes to one hour when milk was exposed to sunlight, depending on the intensity of the light and the mixing of the milk. Kon and Watson (1936) considered the reaction was due mainly to visible radiation of short wave length (white and blue) although ultraviolet radiation was also probably active. They reported that milk exposed to sunlight on the doorstep for half an hour and then kept for one hour in the dark lost its original antiscorbutic properties.

Buruiana (1937) stated that one factor responsible for the reduction of methylene blue in milk exposed to sunlight was the oxidation by catalytic dehydrogenization of the ascorbic acid present in the milk. Henry and Kon (1938) found sterilized milk to behave normally on exposure to light, giving no titration with indol-phenol reagent.

c. Effect of feed on the ascorbic acid content of milk. The effect of various feeds on the ascorbic acid content of milk has been studied by many investigators. Riddell and associates (1935) and Riddell et al (1936) could find no significant difference in the ascorbic acid content of milk due to feeds. Anderson (1936a) found that feeding cabbage, which was high in ascorbic acid, had no beneficial effects in regards to development of rancid and oxidized flavors. He believed that ascorbic acid was not the factor responsible for good flavored milk, whereas, Brown and associates (1937a) feeding one quart either of lemon juice or tomato juice or one gram of crystalline ascorbic acid per day, found the milk to resist oxidized flavor development. They concluded that ascorbic acid in rations of dairy cows might reduce or entirely eliminate the susceptibility of milk to oxidized flavor development.

Brown and associates (1939) found the addition of ascorbic acid to the ration did not increase materially the ascorbic acid content of the milk. They believed the ascorbic acid supplement acted in some manner other than by directly increasing the ascorbic acid content of the milk.

Garrett et al (1940) found that feed had no influence on the amount of ascorbic acid secreted in the milk. Beck and associates (1939) found that the mean ascorbic acid content of fresh milk was practically the same in milk susceptible to oxidized flavor as in milk that was non-susceptible. No relation was found between the amount of ascorbic acid in the original milk or between the amount lost during storage and the development of oxidized flavor.

Trout and Gjessing (1939) found the ascorbic acid content of commercial bottled winter milk to be lower than that of spring, summer or fall milk.

Brown et al (1939) reported that when ascorbic acid was fed to cows on a ration low in carotene there was a slight increase of carotene in the milk produced. Guthrie and associates (1939) found the ascorbic acid content of milk from different cows varied greatly, but remained relatively constant from milking to milking in individual cows.

Garrett et al (1938) found a correlation between ascorbic acid and flavor after three days storage and believed ascorbic acid to help stabilize flavor.

Woessner and associates (1939) found the milk from Brown Swiss cows to contain more ascorbic acid than the milk from Holsteins, Jerseys or Guernseys. His work also indicated that homogenization tended to destroy the ascorbic acid in milk.

d. Effect of adding ascorbic to the milk. Chilson (1935), Dahle and Palmer (1937), Dahle (1938), Greenbank (1936) and Guthrie et al (1939) have all shown that the addition of pure crystalline ascorbic acid to milk at the rate of 20 to 60 milligrams per liter retarded or entirely prevented the development of oxidized flavor.

Effect of carotene on oxidized flavor. Since carotene has reducing properties and reducing substances in milk tend to prevent an oxidizing reaction, it would be expected that carotene would exert a protective action on the flavor of milk.

Anderson (1936a) (1936b) found the feeding of carrots to be effective in improving the flavor of milk toward both oxidized and rancid flavors. The feeding of pure vitamin A was not as effective as was carotene in the form of carrots. However, Dahle and Palmer (1937) found that the addition of carotene to milk did not prevent the occurrence of the off-flavor. Stebnitz and Sommer (1937b) stated there was no relationship between the carotene content, as evidenced by the color of the fat, and the stability of fat toward oxidation. Dahle (1938) found that beta carotene mixed with fat and then emulsified in milk did not prevent the occurrence of off-flavor.

Anderson et al (1937) found that enriching the ration of cows producing either rancid or oxidized milk with plant materials of high carotene content, enabled those animals to produce milk again of very good flavor after a period of ten to fifteen days. They believed that rancid, oxidized and insipid flavors had their origin in carotene deficient rations.

Brown and associates (1937a) observed that green feeds as well as fresh hay produced from it, which are high in carotene, contained considerable amounts of reducing substances which tended to prevent or decrease the intensity of oxidized flavor. Anderson (1936b) found machine cured alfalfa was more effective in reducing oxidized flavor than was field cured alfalfa. Dahle (1938) found very little oxidized flavor occurred after molasses alfalfa silage was fed.

Garrett and associates (1938) observed a positive correlation of 0.7339 between color and first day flavor and a correlation coefficient of +0.6039 between first day color and third day flavor, showing that carotene did help stabilize flavor. Garrett et al (1939) observed that milk of high yellow color tended to lose less of its flavor on storage than did milk of lower color.

Beck and associates (1939) observed a relationship between color intensity of milk fat, as produced by different breeds, and the development of oxidized flavor, with the defect being more prevalent in milk which was below breed average in fat color intensity. The development of oxidized flavor in raw milk was effectively prevented by feeding as little as 206 milligrams of carotene per cow daily. Brown and associates (1939) added a carotene supplement to the ration at the rate of one-half pound (containing 350 milligrams of carotene in oil) per cow which rendered the milk nonsusceptible to metal induced oxidized flavor. Supplementing the ration with carotene increased the carotene content of the milk. However, when cows were placed on a low carotene ration the milk did not develop an oxidized flavor spontaneously, indicating that some other factor besides a low carotene content was responsible for naturally occurring oxidized flavor. They believed that the carotene must be in solution in the fat in order to affect the susceptibility of the milk.

Garrett, Hartman and Arnold (1939) and Garrett and Bender (1940) compared the flavor of milk produced on molasses grass silage, which was high in carotene, with the flavor of milk produced on beet pulp and corn silage, which were low in carotene and found the milk produced on the grass silage to be of superior initial flavor, held their good flavor longer in storage and withstood the destructive effects of soluble copper more than milk produced on either corn silage or beet pulp.

<u>The relation of enzymes to oxidized flavor.</u> Kende (1931) postulated that oiliness in whole milk was due to the enzyme "oleinase" activated by an exogen or endogen contamination with heavy metals.

Chilson (1935) showed that the enzyme was in the skim milk phase by developing an oxidized flavor in a milk remade from raw skim milk and cream heated to 170° F. for 10 minutes. The addition of copper was necessary in some cases to cause the off-flavor, showing the enzyme alone was not a sufficient catalyst.

Dahle and Palmer (1937) found that when susceptible cream was mixed with normal skimmilk the resulting milk was generally susceptible to oxidative changes. They found the enzyme to be inactivated at temperatures of 165° and 168° F. and concluded that the causative factor was destroyed, rather than that reducing substances were formed.

Webb and Hileman (1937) presented evidence that the mechanism of oxidation by oleinase was entirely different from the mechanism of the catalysis by copper, since the former did not involve high oxidation-reduction potentials, while the latter did.

Chilson (1935) believed that the enzyme in the skimmilk was the major factor involved in causing the destruction of ascorbic acid in pasteurized milk. Sharp and associates (1936) found the accelerating effect of soluble copper on the development of the oxidized flavor and the oxidation of ascorbic acid to be much more pronounced in the presence of the active enzyme.

Anderson (1937) (1939) found that the addition of small amounts of pancreatic enzyme to milk prevented the development of oxidized flavor. The enzyme was used in concentrations varying from 1 part of enzyme powder to 40,000 to 80,000 parts of milk.

Relation of various constituents of milk to oxidized flavor:

a. Effect of fatty constituents. Early investigators were of the opinion that the oxidation of fat was the cause of oxidized flavor in milk. Whitehead (1930) treated skim milk with the sodium salts of palmitic and oleic acid and found the sample containing the sodium oleate to cause the reduction of methylene blue within a short time, whereas sodium palmitate had no effect. He believed that the reduction of methylene blue, the development of off-flavors by sunlight, and the oxidation of unsaturated fats were closely related.

Stebnitz and Sommer (1937a) found the development of peroxides in butterfat ordinarily preceded the appearance of a tallowy flavor. Henderson and Roadhouse (1934) found that direct sunlight markedly increased the susceptibility of milk fat to oxidation and that an increase in iodine number was accompanied by a shorter induction period. Stebnitz and Sommer (1937b) found considerable variation in the stability toward oxidation of the butterfat from different cows. The amount of linoleic acid rather than the oleic acid was found to govern the stability of butterfat. Dahle and Palmer (1937) found a decrease in iodine number of the fat in the samples which developed an oxidized flavor.

Brown and associates (1937) stated that it was possible that in highly oxidized milk the butterfat might become oxidized, whereas, in mildly oxidized milk only the substances of the adsorbed film of the fat globule were oxidized. There was no measurable change in iodine number of the fat as a result of the development of oxidized flavor.

Tracy and Ruehe (1931) found skimmilk to obtain a metallic flavor on addition of copper, but when fat was present a tallowy flavor resulted, the intensity of flavor increasing with the fat content. Roland et al (1937) noted that the highest percentage of oxidized flavor occurred in high-fat, premium-quality milk. Fifty per cent of the samples having a fat content between 4.0 and 5.1 per cent showed an oxidized flavor, whereas, only 4.8 per cent of the samples having a fat content from 3.6 to 3.9 per cent showed the defect. Roland and Trebler (1937) found that a variation of one per cent fat in the range of whole milk could be detected by a significant change in flavor score. Hammer and Cordes (1920) found sunlight to have a greater effect on low fat milk than high fat milk.

Evidence has been presented by Thurston et al (1935) indicating that the phospholipid portion of the milk, rather than the butterfat, was the substance which became oxidized when an oxidized flavor developed. They found that when the adsorbed layer was removed by washing and the butterfat redispersed in fresh skimmilk, no oxidized flavor could be detected in the remade milk. By dispersing tallowy butterfat in fresh skimmilk a flavor different from the typical oxidized flavor resulted. The intensity of the flavor was greater in cream, buttermilk and butter than in skimmilk or whole milk.

Chilson (1938) also believed the typical oxidized flavor of whole milk to be due to an oxidation of lecithin or similar substances adsorbed on the fat globule, rather than to oxidation of the true fat, while a tallowy flavor was due to the oxidation of oleic acid of the true fat.

Thurston and associates (1936) suggested that the protective effect of vigorous agitation, freezing and thawing, and homogenization against oxidized flavor was due to some realignment of the material adsorbed on the fat globule, with lecithin being undoubtedly concerned in this realignment. If the lecithin were transferred from the adsorbed layer on the fat globule to the plasma by these treatments, it would indicate that lecithin, while in the adsorbed layer, was readily oxidized to give rise to oxidized flavor, whereas lecithin dispersed in the plasma was not oxidized in this manner.

Roland and Trebler (1937) found mechanical separation of milk produced a marked decrease in its sensitivity to copper-induced oxidized flavor as evidenced by tests made by recombining cream and skimmilk. They attributed this to the removal of lecithin or related substances by the separator or a change in their distribution between the fat and aqueous phase.

Thurston (1938) found that the dispersion of purified lecithin, which became oxidized during purification, in skimmilk yielded a mixture having a typical oxidized flavor.

Dahle and Palmer (1937) working with mixtures of lecithin free fat and susceptible skimmilk produced an oxidized flavor upon the addition of copper, whereas, mixtures of butter oil and normal skimmilk showed little or no flavor development. Their results indicated that it was possible for oxidized flavor to occur in the absence of lecithin in the fat globule membrane providing the enzyme oleinase was present. Josephson and Doan (1939) gave supporting evidence that phospholipids were responsible for tallowy flavor.

Brown and associates (1937b) while studying the effect of metal-induced oxidized flavor on the iodine number of the milk fat concluded that the lecithin of the adsorbed film was the constituent oxidized. Cephalin, however, might also be responsible. They did not find any changes in the iodine number of the lecithin or find any measurable change in the iodine number of the milk fat when milk became oxidized. Swanson and Sommer (1940) found reductions of 44.41 and 30.89 per cent in the iodine number of the phospholipid fraction upon the development of oxidized flavor.

Fox (1937) found only a slight relationship between the per cent lecithin in the fat of the milk and the intensity of the oxidized flavor which developed in that milk. The oxidized flavor developed over the entire range of lecithin percentages in all degrees of intensity. Beck and associates (1939) obtained similar results.

b. Effect of non-fatty constituents. The development of off-flavors in milk due to sunlight have recently been attributed to changes in the non-fatty constituents of milk. Doan and Meyers (1936) believed that the burnt flavor caused by sunlight apparently had its source in the casein-free milk and albumin-free serum of the milk. Weckel and Jackson (1936) believed that the activated flavor originated in or was closely associated with the protein fraction of milk. Albumin obtained from milk unduly exposed to radiation possessed a more intense activated flavor than did the casein. The flavor was also produced in the filtrates after removal of casein and albumin and was believed to be due to the soluble minor proteins and to adsorption from casein and albumin.

Flake and associates (1939) found a one per cent solution of gelatin to develop an activated flavor on exposure to radiation. They believed that the activated flavor and the burnt flavor produced by sunlight to be identical or practically so.

II. Relation of Processing to Oxidized Flavor Development

Effect of metals on oxidized flavor development. The catalytic effect of copper on the development of oxidized flavor in milk is well known. Thurston (1935) and Roadhouse and Henderson (1935) showed that copper and iron must be in solution in order for it to catalyze the development of oxidized flavor. Brown et al (1936) and Josephson and Doan (1939) found that copper was more effective in causing an oxidized flavor when added after pasteurization. Thurston (1935) and Brown et al (1937a) and Greenbank (1936) found that considerably more ferrous iron than copper was required to produce oxidized flavor. Greenbank (1936) found that ferric iron, which was an oxidizing agent, was an inhibitor of oxidized flavor, especially in the higher concentrations.

Tracy et al (1933), Thurston (1935), Webb and Hileman (1937) and Gould and Sommer (1939) have shown that the addition of copper and iron to milk caused a rise in the oxidation-reduction potential. Webb and Hileman (1937) believed that copper increased the oxidation-reduction potential of the milk to a point sufficiently high to bring about a change in some milk constituent. Thurston (1935) found tin and aluminum salts to lower the oxidation-reduction potential of milk.

Roadhouse and Henderson (1935) stated that metallic salts gave a taste properly designated as metallic, some of them, however, acted as catalysts, and hastened the oxidation of the milk fat.

Hunsiker et al (1929) found that Allegheny metal, tin and heavily tinned copper had no effect on the flavor of milk. Monel metal, Enduro, Ascoloy and nickel had only a slight effect on flavor, while tinned iron, copper, galvanized iron, iron and zinc affected milk flavor the most.

Guthrie and associates (1931) found copper and copper alloys produced oxidized flavor in milk. Chromium alloys caused slight oxidized flavor at times, whereas, chromium-nickel alloys, pure aluminium, glass enamel and carefully tin-plated metals produced no oxidized flavor in milk.

Gould and Sommer (1939) showed that copper raises the point to which milk must be heated in order to prevent an oxidized flavor, and also raised the temperature at which sulphide liberation began. Ferrous iron was found by Gould (1939) to have little effect on sulphide liberation or the temperature at which milk must be heated to prevent oxidized flavor.

Josephson and Doan (1939) found copper to retard sufhydryl formation during heating and oxidized those which were formed after heating.

<u>The relation of oxidation-reduction potentials to oxidized flavor.</u> Sommer (1938) stated that the intensity of oxidizing conditions in milk or other liquids could be measured electrically and expressed in terms of oxidation-reduction potential. Tracy, Ramsey and Ruehe (1933) showed that freshly drawn milk had a normal tendency toward reduction. The addition of copper moved the potential toward the oxidation side, whereas, incubation of milk usually caused a rapid drop in potential. They believed that oxidation-reduction potentials were related to fat oxidation in dairy products.

Thurston (1935) found that the oxidation-reduction potentials of milk treated with copper were always higher than the controls, and the potentials of tin and aluminum treated samples were always lower than the controls. The oxidation-reduction potentials were of no practical value in predicting samples which had a tendency to develop oxidized flavor. Greenbank (1936) found that milk with the lowest potential was best, but the difference in potentials were not great enough to indicate that this was a property which controlled the development of the flavor.

Webb and Hileman (1937) determined the oxidation-reduction potentials of milk from individual cows and concluded that the absolute value of the oxidation-reduction potential of unmixed milk pasteurized in glass had no relation to the degree of oxidized flavor which developed. Oxidized flavor developed in some samples when the oxidation-reduction potential was very low. Evidence indicated that the flavor of mixed milk in the winter was directly related to the oxidation-reduction potential. The development of oxidized flavor in milk by the addition of copper was due to or accompanied by an increase of the potential to a point sufficiently high to bring about a change in some milk constituent. Summer milk was able to resist the development of oxidized flavors even in the presence of a high oxidation-reduction potential.

Fox (1937) found little or no relationship to exist between the potential of individual milk samples and the development of oxidized flavor.

Greenbank (1938a) suggested a method of determining milk which would become oxidized. A small amount of copper was added to milk and the increase in potential was determined. Unusual increases in potential indicated samples which might become oxidized. In another work (1938b) he stated that thermal inhibition of the flavor was shown to act through a lowering in oxidation-reduction potential, and the inhibition of the flavor by green feed was paralleled by a decrease in potential and an increase in poising action.

Gould and Sommer (1939) showed that the oxidation-reduction potential of milk heated momentarily remained unaltered until temperatures of 80° C. or above were used. At these temperatures the potential showed a definite trend toward a more negative level, the higher the temperature the lower became the potential. Changes in the oxidation-reduction potential were found to occur coincidental with the appearance of the cooked flavor. When a 30 minute holding period was used the first appreciable lowering of the potential occurred at a temperature of 72° C. They attributed this decrease to the liberation of sulphides within the milk.

Josephson and Doan (1939) confirmed the work of Gould and Sommer and further reported that raw milk and milk heated under 180° F. exhibited a rise in potential upon storage, whereas, samples heated above 180° F. showed no rise in potential.

Whitehead (1931) reported some interesting work on the effect of sunlight on the oxidation-reduction potential of milk. He found that when milk was exposed to sunlight, considerable changes in Eh were soon observed coincident with the decoloration of methylene blue. The time required to bring about the change and also the extent of the change varied considerably with different milks, and with the intensity of sunlight. The form of the curve obtained with whole milk exposed to sunlight was quite different from that obtained by plotting oxidation-reduction potentials with time in milk of high bacterial counts. The lower limit of the Eh value of whole milk was found to be about -0.20 volts. When skimmilk was treated in the same manner little change in Eh occurred in some samples, whereas, in others it fell to about zero and then showed a tendency to rise again to an Eh of about 0.20 volts.

<u>Effect of heat on oxidized flavor.</u> Frazier (1928) noted that pasteurized samples usually developed a tallowy flavor and odor before raw samples. This has been confirmed by Sharp et al (1936), Dahle and Palmer (1937), and many other investigators.

Frazier (1928) found that when milk was sterilized the defect appeared more slowly. Guthrie and Brueckner (1933) showed that pasteurization temperatures of 160° F. and higher for a period of 30 minutes decreased and even prevented the tendency for the development of oxidized flavor. Dahle and Palmer (1937) stated that it would appear reasonable to conclude that the causative agent was destroyed rather than that reducing substances were formed. Thurston (1938) believed that heating from 165° to 168° F. probably resulted in the destruction of the causative enzyme. Greenbank (1936) and Dahle (1938) also noted the inhibiting effect of high temperatures on the development of oxidized flavor in milk.

Greenbank (1938b) reported that the thermal inhibition of the flavor was shown to act through a lowering of the oxidation-reduction potential.

Sharp et al (1936), Greenbank (1936), Guthrie and associates (1939) and Gould and Sommer (1939) have noted that the undesirable effect of copper was much less evident in the milk heated to the higher temperatures.

Chilson (1935) found that when skimmilk which had been held at 170° F. for 10 minutes was mixed with raw cream to form a 4.0 per cent milk no oxidized flavor occurred, but when the cream was heated to 170° F. for 10 minutes an oxidized flavor developed.

Gould and Sommer (1939) found a relation between oxidized and cooked flavors. They pointed out that the apparent coincidence between the temperature necessary to prevent the oxidized flavor in milk and the temperature required to cause a cooked flavor indicate the possibility that the failure of highly heated milk to show an oxidized flavor might be partially due to the covering up effect of cooked flavor, and secondly to the formation of reducing substances which were closely allied with the cooked flavor.

The addition of copper necessitated a rise in temperature in order to prevent oxidized flavor and to cause sulfhydryl liberation. Josephson and Doan (1939) confirmed these results.

Effect of light and radiation on oxidized flavor. The accelerating effect of light on oxidized flavor development in milk has been studied by Hammer and Cordes (1920), Frazier (1928), Thurston (1935), Trout (1937), Doan and Meyers (1936), Whitehead (1930) and many others. Hammer and Cordes (1920) found an exposure of ten minutes to sunlight was sufficient to cause an off-flavor while an exposure of 45 minutes was sufficient to cause a definite tallowy flavor. These flavors did not develop when the milk was placed in brown glass bottles during exposure to sunlight.

Frazier (1928) exposed milk to diffused light and found a characteristic cardboard flavor and odor to develop. Skimmilk did not develop the cardboard flavor. He pointed out that there was a difference in the flavor caused by exposure to light and the flavor resulting from mixing tallowy fat in fresh skimmilk. Tracy and Ruehe (1931) showed that a tallowy flavor developed when milk was exposed to diffused light and a burnt flavor when the milk was exposed to sunlight. They found the defect was more noticeable in skimmilk than in whole milk and believed that the defect was due to an action upon the milk serum rather than the fat. Davies (1931) stated that the rays which affected milk in bottles were those which were chemically active and capable of passing through clear glass. Homogenized milk was found to be more susceptible to off-flavor development than untreated milk when exposed to light.

Whitehead (1930) gave evidence supporting the theory that the reduction of methylene blue in milk by sunlight was an oxidation-reduction phenomenon in which unsaturated fats were oxidized and methylene blue acted as a hydrogen acceptor.

Henderson and Roadhouse (1934) found direct sunlight markedly increased the susceptibility of milk fat to oxidation. Stebnitz and Sommer (1937a) observed that the end of the induction period during the oxidation of butterfat was marked by a rapid bleaching of the color, and that light, especially ultra-violet, exerted a marked catalytic effect on the oxidation of butterfat. Aluminum foil wrappers were found to exclude the light entirely and prevented the oxidation of the butterfat. Dark green and dark red transparent wrappers were more effective than other colors in preventing the reaction. Doan and Meyers (1936) found blue and green colored paper bottles or blue and green cellophane wrappers on paper bottles reterded the development of tallowiness and burnt flavors. They further reported that when whole milk and skimmilk were exposed in clear glass they acquired a burnt flavor, whereas, when exposed in paper bottles they commonly acquired a milk tallowy flavor. Milk in the paper bottles withstood 10 minutes more exposure than did milk in the clear glass bottles without exhibiting an off-flavor.

Weckel and Jackson (1936) distinguished between oxidized flavor and the flavor due to light. According to them the activated flavor originated in or was closely associated with the protein fraction of milk. When albumin was separated from milk unduly exposed to radiation it possessed a more intense activated flavor than the casein. The presence of the flavor in the filtrate after the removal of casein and albumin was believed to be due to the effect of radiation on the minor soluble proteins and to adsorption from casein and albumin. Radiation might possibly exert an effect upon a reactive substance in whole milk not represented by any one of the constituents fractionated and studied.

Thurston (1938) also noted a difference in the flavor produced by sunlight and the so-called oxidized flavor found in spontaneous milk or in copper treated milk.

Dahle and Palmer (1937) found sunlight caused the off-flavor to occur in samples in which the free oxygen was replaced by nitrogen.

Flake, Weckel and Jackson (1939) found preheating to temperatures of 150° - 200° F. resulted in a slightly greater intensity of the activated flavor in the milk subsequently exposed to radiation. The addition of various amounts of salts commonly found in milk had no effect on the intensity of the flavor noted when the mixtures were irradiated. The addition of a small amount of hydrogen or calcium peroxide either before or after irradiation caused a marked decrease in intensity of flavor. The activated flavor was removed by the addition of copper followed by bubbling air through the milk. The flavor was inhibited by replacing the oxygen of milk with nitrogen. Colored glass filters which eliminated all wave lengths less than 4600 A° were effective in preventing development of activated flavor.

Effect of gases, aeration and vacuum on oxidized flavor. Hammer and Cordes (1920) found that exposure to air apparently had some influence on the development of tallowiness in milk. Mattick (1927) expressed the idea that the reaction was, to a large extent, dependent on free access to oxygen in the molecular state.

Dahle and Palmer (1937) replaced the dissolved oxygen in the milk with nitrogen and found it prevented or reduced the development of the off-flavor. However, sunlight caused the off-flavor in these samples. When oxygen was passed through samples heated to 180° F. from which the oxygen had been removed previously, the flavor failed to develop. Dahle

(1938) showed, however, that oxidized flavor would develop in milk deprived of its free oxygen by nitrogen when copper was added. Flake and associates (1939) found that the production of activated flavor either by sunlight or artificial ultra-violet radiation was inhibited when nitrogen replaced the natural gas balance exisiting in the milk.

Greenbank (1936) found aeration increased copper tolerance of milk by four times. Aeration plus pasteurization gave greater protection than aeration alone. When oxidized flavored milk was aerated some of the flavor was removed. They believed that in this case the flavor was probably adsorbed by the fat or protein which made it more difficult to oxidize.

Kon and Watson (1936) and Guthrie et al (1939) found that oxidation of ascorbic acid did not occur in oxygen free milk even in the presence of sunlight.

Effect of agitation, freezing and thawing on oxidized flavor. Thurston and associates (1936) showed that vigorous, prolonged agitation of milk at low temperature had a marked effect in reducing or eliminating the development of oxidized flavor even when copper was added in sufficient quantities to cause the off-flavor in untreated milk. Such agitation was found to cause some movement of the lecithin from the adsorbed layer on the fat globule to the plasma. Freezing and thawing was found to have the same effect.

Thurston (1938) suggested that this reduction in the development of oxidized flavor in milk agitated or frozen might possibly be explained by a reduced fat surface.

The effect of methods of sterilizing equipment on oxidized flavor. Dahlberg and Carpenter (1936) studied the influence of methods of sterilizing equipment on oxidized flavor development and found that when hot water sterilization was employed the raw milk and hot milk which passed through the equipment, after the first 50 pounds had been passed through, kept well for three days without the development of oxidized flavor, whereas the first 25 pounds through the equipment developed a trace of oxidized flavor in one day and was intense in two days. When chlorine sterilization was used the first 25 pounds of milk passed through the equipment was almost unfit to drink within one hour due to a coal tar flavor. There was a tendency for the milk processed in equipment sterilized with chlorine to be poorer in flavor than other milk after a storage of three days.

The effect of antioxidants on oxidized flavor. The use of oat flour as an antioxidant in milk has been studied by Dahle and Palmer (1937), Dahle (1838), Mueller and Mack (1939) and Garrett (1940). Dahle (1938) observed that the addition of 0.1 to 0.2 per cent of oat flour reduced the development of oxidized flavor and 0.3 per cent was sufficient to prevent its development. Mueller and Mack (1939) using copper contaminated milk noted the anti-oxidative properties of cereal flour at the beginning of the second day of storage. Garrett (1940) found oat oil to be more effective as an anti-oxidant than oat flour.

The antioxidative properties of ascorbic acid, when added to milk in crystalline form, has been observed by Chilson (1935), Dahle and Palmer (1937), Dahle (1938) and Guthrie et al (1939).

Dahle and Palmer (1937) and Dahle (1938) found hydroquinone to be an effective antioxidant when added at the rate of two p. p. m. Greenbank (1936) found that formaldehyde did not have much effect upon the development of oxidized flavor, whereas, hydrogen peroxide prevented the development of the flavor. Hammer and Cordes (1920) showed that small amounts of commercial lactic acid did not have any important influence on the development of tallowiness. Dahle (1938) found malic acid to intensify the off-flavor.

Anderson (1937) (1939) showed that the addition of small amounts of pancreatic enzyme to milk prevented the development of oxidized flavor when added at the rate of one part of enzyme to 40,000 to 80,000 parts of milk.

III. The Effect of Homogenization upon the Properties of Milk

a. Physical properties. The fact that the homogenization of milk reduced the size of the fat globules and prevented creaming has been shown by Weigner (1914), Baldwin (1916), Doan and Swope (1927), Smallfield (1929) and Halloran and Trout (1932). Weigner (1914) found the diameter of the fat globules in normal milk to average 2.9 microns, while those in homogenized milk averaged 0.27 microns, whereas Baldwin (1916) reported values of 5.0 to 6.0 microns for normal milk and 1.0 to 2.0 microns for homogenized milk. Similar results have been obtained by Halloran and Trout (1932) and by Doan (1938). Halloran and Trout (1932) showed that pressures of 1,500 pounds per square inch at 90° F. or at 145° F. was usually sufficient to prevent the formation of a cream layer. Trout and associates (1935) found that at any given pressure the reduction in the size of the fat globule was greater when the milk was homogenized at 145° F. than when processed at 90° F. Whitaker and Hilker (1937) confirmed these results and further showed that milk with hardened fat when homogenized at 50, 60 and 70° F. failed to show reduction in the size of the globules. However, some reduction in size was noticed at 80° F.

Halloran and Trout (1932) and Doan and Minster (1933) found that the protein stability toward alcohol was decreased by homogenization. The latter investigators attributed this to the greater amount of adsorbed casein; such casein being fixed and was in the first stages of coagulation.

Doan and Minster (1930) stated that the surface tension was increased slightly in homogenized milk, while Halloran and Trout (1932) found homogenization increased the surface tension of pasteurized milk but lowered the surface tension of raw milk. Doan and Minster (1933) confirmed these results and attributed the decrease to the presence of free soluble fatty acids.

The fact that homogenization lowers the curd tension of whole milk has been shown by Smallfield (1929), Halloran and Trout (1932), Theophilus and associates (1934) and Doan (1938). Whitaker and Hilker (1937) have shown that this reduction in curd tension occurs whenever the homogenization temperature was 90° F. or above, with more effective reduction resulting by cooling down to 90° F. than by heating up to this temperature. Theophilus and associates (1934) found that homogenization at pressures of 500, 1,000 or 2,000 reduced the curd tension of milk approximately 25, 46 and 53 per cent respectively, with the greatest reduction occurring in milk with high original curd tension.

Hollingsworth (1931), Trout (1933), Babcock (1934b), Hood and White (1934) and Doan (1938) found that homogenization presented a problem in securing a satisfactory Babcock fat test; a lower test was usually obtained when the milk was homogenized. A plug at the base of the fat column has been noticed by Trout (1933) and Hood and White (1934), the removal of which necessitated the use of sulphuric acid at a lower specific gravity and at more carefully controlled temperature than those commonly employed.

No important changes in specific gravity could be found in milk due to homogenization either by Weigner (1914), Trout (1933) or Babcock (1934b).

A brown to yellow sediment in homogenized milk has been noticed by Trout and Halloran (1932), Babcock (1934a) and Charles and Sommer (1934). Babcock (1934a) found the sediment to consist largely of leucocytes and epithelial cells, and later (1939) reported there was no relation between temperature and pressure of homogenization and the formation of sediment. Trout and Halloran (1933) analyzed the sediment and found its composition to be approximately 72 per cent water, 11 per cent fat and 17 per cent solids not fat. Clarification is used as a means of eliminating the sediment.

Weigner (1914) and Doan and Minster (1933) noticed an increase in viscosity when milk was homogenized which they explained was due to increased adsorption of substances on the fat. Trout and associates (1935) reported decreases in the viscosity and foaming ability when milk was homogenized raw but these were increased when the milk was pasteurized before homogenization.

Several workers have shown that homogenized milk and cream are difficult to churn and whip. Clayton (1935) attributed these phenomena to the increased adsorption of milk protein, notably casein, on the fat globule. By using viscosity measurements, Weigner (1914) calculated that of the casein in milk, 2.27 per cent was adsorbed in ordinary milk and 25.2 per cent in the homogenized milk, based on the assumption that only casein is adsorbed and that the mean thickness of the adsorbed layer is 6.3 millimicrons.

<u>b. Chemical properties.</u> The most noticeable chemical change in milk caused by homogenization is the change in the acid degree of the fat re-

sulting in a rancid flavor. Dorner and Widmer (1932) and Halloran and Trout (1932) working independently discovered almost simultaneously that a rancid flavor and an increase in acidity occurred in raw milk within one-half to four hours after homogenization, with the acidity and degree of rancidity increasing with the pressure. Sharp and de Tomasi (1932) showed the increase in acidity to range from 0.04 to 0.08 per cent.

The development of rancidity and the increase in acidity is attributed to the action of lipase on the increased fat surface by Dorner and Widmer (1932), Halloran and Trout (1932) and Doan and Minster (1933). Sharp and de Tomasi (1932) working with homogenized cream substrate concluded that the increased activity of lipase cannot all be explained on the basis of increased surface otherwise they would have obtained greater increases in acidity. Pfeffer, Jackson and Weckel (1938) also believed the increased activity of lipase was not due entirely to decrease in fat globule size. Doan (1938) stated that homogenization probably caused the destruction of the adsorbed layer on the normal fat globule and allowed the lipase to come in contact with the fat.

The causative agent of rancidity was inactivated by heating to a temperature of 131° F. as shown by Dorner and Widmer (1932). Doan (1933) found a flash heating of 147° F., a temperature of 134° F. for 15 minutes or a temperature of 132° F. for 30 minutes would prevent rancidity in milk homogenized at 2,000 pounds. Trout and associates (1935) found subsequent pasteurization did not decrease the acidity resulting from homogenization. The acidity remained constant regardless of the pressure when the milk was pasteurized prior to homogenization. Homogenized raw skimmilk showed no increases in acidity.

Babcock (1934b) found the optimum temperature of homogenization for the development of rancidity in raw milk to be from 30 to 40° C. Dorner and Widmer (1932) and Whitaker and Hilker (1937) showed that when the milk was homogenized while the fat was in a hardened condition rancidity did not develop.

Dorner and Widmer (1932) found that distinctly alkaline milk became rancid rapidly after homogenization, but acidified milk became rancid only slowly. He further concluded that the agent of rancidity was not contained in the fat or in the whey. Doan (1933) and Pfeffer, Jackson and Weckel (1938) believed that the lipase was to be found primarily in the milk plasma rather than being associated with the fat.

Gould and Trout (1936) found no appreciable difference in the Reichert-Meissel number, the Polenski number, the refactive index, or in the acid degree of the fat when pasteurized milk was homogenized. They found the acid degree of the fat to increase four to six times within a few minutes when raw milk was homogenized, with the greatest daily change occurring during the first 24 hours when an increase of 1,652 per cent was noted.

Woessner and associates (1939) observed that homogenization tended to destroy the ascorbic acid in milk.

<u>The effect of homogenization on oxidized flavor.</u> Tracy, Ramsey and Ruehe (1933) and Doan and Minster (1933) were perhaps the first to show that homogenization retarded the development of oxidized flavor in milk which had been contaminated with copper. Their results have been confirmed by Thurston and associates (1936), Dahle and Palmer (1937), Ross (1937), Trout and Gould (1938) and others. The ability of homogenized milk to withstand oxidative changes depends upon the pressure of homogenization

as shown by Ross (1937), who found that pressures of 500 or 1,000 pounds could not be depended upon to prevent the development of oxidized flavor, while at pressures of 1,500 pounds no samples developed the defect. Samples containing copper added before homogenization did not develop the defect within 96 hours when pressures of 1,500 pounds were employed. Almost identical results were obtained when the copper was added after homogenization.

Trout and Gould (1938) found that pressures of 1,500 pounds had a marked effect in stabilizing the flavor. Pressures of 2,500 and 3,000 were sufficient to inhibit the development of the oxidized flavor when copper was added at the rate of 5.0 p. p. m. prior to homogenization. A pressure of 3,000 pounds inhibited oxidized flavor development in milk contaminated with 2.5 p. p. m. of copper added after processing, but was ineffective when 5.0 p. p. m. of copper were added. Ross (1937) treated cream in the same manner as he did milk and obtained almost identical results. Trout and Gould (1938) supplemented these results by showing that homogenization stabilized the flavor of milk containing from 2.0 to 10.0 per cent fat. However, the process was ineffective when fat was present in inappreciable quantities as in skimmed milk. They also found the effectiveness of homogenization was not lessened by the addition of 1.0 to 4.0 per cent of serum solids to the milk before homogenization.

Ross (1937) observed that homogenization did not destroy the oxidized flavor when the flavor had developed before the milk was homogenized.

Holm, Greenbank and Deysher (1925) found that homogenization improved the keeping quality of milk powder by increasing the ability of the fat to withstand oxygen adsorption. Bell (1939) showed that homogenization caused condensed milk to be more resistant to the changes which result in oxidized flavor.

Theory of protective action of homogenization. The mechanism by which homogenization retards oxidized flavor development is not known. Ross (1937) gave a hypothesis based on the assumption that oxidized flavor was caused by enzymic action on the fat globules. Under this assumption the finely divided fat globules, resulting from adequate homogenization, are surrounded by a film which protects them from enzymic action, thus preventing the development of oxidized flavor. Dahle (1938) stated that it was thought that the process added a fairly heavy film on the surface of the globule which protected it from oxidation.

Tracy, Ramsey and Ruehe (1933) thought the retardation of oxidation to have been due to certain physical changes in the milk which might have made the oxidized flavor less detectable.

Thurston (1938) stated that the most likely theory to explain the non-development of oxidized flavor in homogenized milk was the increased adsorption of protective protein on the surface of the fat globules.

Effect of light on homogenized milk. That homogenized milk is more sensitive to sunlight than unhomogenized milk has been demonstrated by Davies (1931), Hood and White (1934), Doan and Meyers (1936), Dahle (1938) and Flake, Weckel and Jackson (1939).

Davies (1931) pointed out that homogenized milk exposed more fat surface than the same sample of natural milk, and, coupled with this was the fact that during atomization the milk had become completely saturated with atmospheric oxygen. Such treatment of milk was likely to lower the protective effect of the hulls or coatings of the fat globules thus rendering the fat more susceptible to deterioration.

This flavor defect due to light has been called sunshine oxidized, tallowy, burnt and activated. The term burnt or activated as suggested by Flake and associates (1939) described the flavor more adequately and seemed more desirable. Dahle (1938) used the term oxidized to describe the flavor defect in homogenized milk after exposure to the sun. Doan and Meyers (1936) found homogenization of whole milk to accelerate the development of tallowy flavor in both glass and paper bottles, but the burnt flavor was not evident. At high pressures of homogenization the degree of tallowiness was greater in the paper bottles than in the clear glass bottles. They also found that homogenization had no noticeable effect on the development of the burnt flavor in skimmilk exposed to light.

Flake and associates (1939) found that a slightly more intense flavor resulted when homogenized milk was irradiated than when milk was irradiated only, or irradiated then homogenized. They suggested that perhaps the parent substance or substences which gave rise to the flavor might be made more available to, or more susceptible to the action of ultra-violet rays and to the sunlight by the homogenization process.

SCOPE OF INVESTIGATION

This study was conducted in an effort to determine if possible the mechanism by which homogenization retards the development of oxidized flavor in pasteurized milk and accelerates the development of rancidity in raw milk. The experiment included a study of the following points:

1. To compare the effect of long storage periods on the flavor and oxidation-reduction potentials of unhomogenized and homogenized, pasteurized milk.
2. To compare the effect of long storage periods on the flavor and the oxidation-reduction potentials of milk when copper was added.
3. To determine the effect of removing the fat globule membrane by churning and washing on the development of oxidized flavor in the milk and the lecithin content of the buttermilk.
4. To study the development of oxidized flavor in milk remade from unhomogenized skim milk and cream, and homogenized skim milk and cream.
5. To compare the vitamin C content of raw, pasteurized and homogenized milk as related to the development of oxidized flavor.
6. To determine the effect of sunlight on the oxidation-reduction potential and flavor in unhomogenized and homogenized milk.
7. To study the development of rancidity in homogenized raw milk.

EXPERIMENTAL PROCEDURE

The milk used in this experiment was obtained at the college creamery, and usually consisted of mixed milk from two or three producers. The quality was similar to that received at the average milk plant, with the butterfat content varying from 3.5 to 4.0 per cent.

Pasteurization was accomplished at a temperature of 143° F. for 30 minutes in an open, stainless steel, 20 gallon cheese vat.

Homogenization was accomplished at pasteurizing temperatures, except for the raw milk which was homogenized at 90° F, with a 200 gallon per hour Cherry-Burrell viscolizer at a pressure of 2,500 pounds, unless otherwise stated. The milk was cooled immediately, bottled and stored at 35° to 40° F.

Oxidation-reduction potentials were determined at various periods throughout the year by means of a Beckman pH meter using a platinum electrode against a saturated calomel cell. The readings were taken when the E. M. F. had become constant and were converted to Eh by adding the E. M. F. to the voltage resistance of the saturated calomel cell which was determined against a normal hydrogen electrode.

In studying the effect of washing the fat on the oxidized flavor, a normal centrifugal cream testing about 35 per cent fat was obtained from pasteurized milk. The fat globule membrane was removed by churning the cream in a glass daisy churn. The butter was washed once with cold water and then redispersed in the original skim milk by homogenizing at 500 pounds pressure. A portion rehomogenized at 2,500 pounds pressure, constituted the sample washed once. The remaining milk was reseparated; the cream rechurned; the butter rewashed and redispersed in its skim milk as before.

A portion rehomogenized at 2,500 pounds pressure constituted the sample washed twice. The remaining milk was treated in the same manner for a third time and constituted the sample washed three times. Care was taken to see that the remade samples had the same fat content as the original milk.

The phospholipid content of the buttermilk from each of the three churnings was determined according to the method suggested by Horrall (1935) except for a few variations in the procedure. The samples of buttermilk were weighed instead of being measured. The dissolved ash was transferred to a 200 ml. volumetric flask and made up to volume. A five ml. portion of the ash solution was transferred to a 100 ml. volumetric flask and made up to volume. The phosphorus content of this solution was determined. The molybdate and stannous chloride solutions were made as suggested by Bodansky (1932).

The recombined milk studied were prepared as follows: cream and skim milk, separated from pasteurized milk, were each divided into two lots, one lot of cream and one lot of skim milk being homogenized at 2,500 pounds pressure and the other lot kept unhomogenized. The following mixtures were then made: 1, homogenized cream plus homogenized skim milk; 2, homogenized cream plus unhomogenized skim milk; 3, unhomogenized cream plus unhomogenized skim milk; and 4, unhomogenized cream plus homogenized skim milk. The remade samples were standardized so as to have the same fat content as the original milk.

Ascorbic acid determinations were made on raw, pasteurized and homogenized pasteurized milk by means of the rapid titration method recommended by Sharp (1939).

The effect of sunlight on the flavor and oxidation-reduction potential of unhomogenized and homogenized milk were studied by exposing the milk in eight-ounce glass jars to direct sunlight. Duplicate samples were exposed in oxidation-reduction cells. These cells were eight-ounce jars fitted with four hole stoppers. Two platinum wire electrodes were inserted into the milk through the holes, with an agar bridge extending from the milk through one hole into a side tube filled with a saturated potassium chloride solution, into which the saturated calomel cell was placed when making connections. The fourth hole in the stopper served only as an air vent so the stopper could be firmly fitted into place.

All milk was studied organoleptically for oxidized flavor by two or three experienced judges. The samples were numbered and scored without the identity of the samples being known. The presence and intensity of the oxidized flavor was indicated as follows: -, no oxidized flavor; ?, questionable oxidized; +, slightly oxidized; + +, distinctly oxidized; and + + +, strongly oxidized. In summarizing flavor scores, each intensity was given a numerical value of 0, 1, 2, 3, and 4 respectively.

Rancidity development was studied by making mixtures of raw milk plus homogenized pasteurized milk, raw milk plus homogenized raw milk, and homogenized raw milk plus homogenized pasteurized milk. These samples were studied organoleptically. The presence and intensity of the rancid flavor was indicated in the same manner as were the oxidized flavors. The increase in titratable acidity was determined by direct titration, using a ten ml. sample of milk and 0.05 normal sodium hydroxide, the increase in acidity being calculated as lactic acid.

RESULTS

The relation of oxidation-reduction potentials to the development of oxidized flavor in unhomogenized and homogenized milk when stored over long periods at a low temperature.

In an effort to determine the relationship between oxidation-reduction potentials and the development of oxidized flavor in unhomogenized and homogenized milk, flavor determinations and oxidation-reduction potential measurements were made immediately after processing and after 1, 3, 7, 10, 14, 17, 21, 28 and 35 days of storage at 35° to 40°F. In this series of experiments the milk was pasteurized at 143° F. for 30 minutes and homogenized at a pressure of 2,500 pounds. The data obtained are presented in tables 1, 2, 3 and 4; with the average results of all trials presented in table 5.

An examination of the data shows there are considerable variations in the oxidation-reduction potentials of different milk immediately after processing. An increase in oxidation-reduction potential occurred when the milk was stored, with the rate of increase being slightly greater in the homogenized than in the unhomogenized milk. The day at which the maximum Eh occurred varied considerably, depending upon the milk. Winter milk did not show very large changes in oxidation-reduction potential during the storage period as compared to the milk produced in the spring or fall. An examination of the average results on all samples presented in table 5 shows the highest potential occurred in both the unhomogenized and homogenized milk after 14 days storage at which time the potentials were 0.4094 and 0.4175 volts respectively. After reaching the maximum the potential decreased until at the end of storage period it was approximately the same as at the beginning of the storage period. The rate of decline

Table 1. The oxidation-reduction potentials and the development of oxidized flavor in unhomogenized and homogenized pasteurized milk after various storage periods. Fall milk, 1938.

Storage (Days)	Oxidation-reduction potentials and flavor when Fall milk was Unhomogenized: Eh	Unhomogenized: Flavor	Homogenized: Eh	Homogenized: Flavor	Unhomogenized: Eh	Unhomogenized: Flavor	Homogenized: Eh	Homogenized: Flavor
	Trial I				Trial II			
0	0.2860	-	0.2800	-	0.2886	-	0.2672	-
3	0.3272	++	0.3392	-	0.2930	++	0.3506	-
7	0.3360	+++	0.3352	-	0.3472	++	0.3466	-
10	0.4322	+++	0.3806	-	0.4099	+++	0.4032	-
17	0.3556	++	0.3243	-	0.4379	++	0.3804	-
28	0.4412	++	0.4812	-	0.4586	+?	0.3422	-
35	(Insufficient sample)				0.2879	+	0.3052	sour
	Trial III				Trial IV			
0	0.2579	-	0.2782	-	0.2764	-	0.2974	-
3	0.2736	+++	0.3216	-	0.2872	-	0.3275	-
7	0.3442	+++	0.3702	-	0.3502	+	0.3784	-
10	0.3496	+++	0.3523	-	0.4223	++	0.4726	-
17	0.4299	+++	0.4212	-	0.3860	++	0.3503	-
28	0.3342	++?	0.3522	-	0.2919	+	0.3584	sour
35	0.2982	++?	0.2906	-	0.3222	+	0.3373	-
	Trial V				Average (5 trials)			
0	0.3504	-	0.3204	-	0.2919	0.00	0.2886	0.00
3	0.3673	+	0.3799	-	0.3097	2.40	0.3438	0.00
7	0.4470	+++	0.4666	-	0.3649	3.40	0.3794	0.00
10	0.3996	++	0.3103	-	0.4027	3.60	0.3838	0.00
17	0.3212	++	0.2912	-	0.3861	3.20	0.3535	0.00
28	0.3376	++	0.3477	-	0.3727	2.60	0.3763	0.00
35	(Insufficient sample)				0.3204*	2.33	0.3110	0.00

*Average of three trials

Table 2. The oxidation-reduction potential and the development of oxidized flavor in unhomogenized and homogenized pasteurized milk after various storage periods. Winter milk, 1939.

Storage (Days)	Oxidation-reduction potentials and flavor when Winter milk was							
	Unhomogenized		Homogenized		Unhomogenized		Homogenized	
	Eh	Flavor	Eh	Flavor	Eh	Flavor	Eh	Flavor
	Trial I				Trial II			
0	0.3008	-	0.2998	-	0.3376	-	0.2586	-
3	0.3550	++	0.3550	-	0.3086	++?	0.3146	-
7	0.3120	+++	0.3136	+?	0.3212	+++	0.3262	-
10	0.3333	+++	0.3239	-	0.3084	++	0.3104	-
21	0.2803	+	0.2762	-	0.3502	++	0.3523	-
28	0.3610	+++	0.3469	-	0.3582	+++	0.3172	-
35		++		bitter		++		bitter
	Trial III				Trial IV			
0	0.3183	-	0.3119	-	0.2800	-	0.2800	-
3	0.3222	+++	0.3211	-	0.3074	++	0.3120	-
7	0.2971	+++	0.3041	-	0.2753	++	0.2809	-
10	0.2822	++	0.2922	+	0.3179	?	0.3179	-
21	0.3646	++	0.3550	-	0.2937	++	0.3082	-
28		+				bitter		bitter
35	(Insufficient sample)				(Insufficient sample)			
	Trial V				Average (5 trials)			
0	0.3140	-	0.3070	-	0.3101	0.00	0.2915	0.00
3	0.2968	++	0.2975	-	0.3180	3.20	0.3200	0.00
7	0.2819	+++	0.2812	-	0.2975	3.80	0.3012	0.40
10	0.3266	++	0.3193	-	0.3137	2.80	0.3127	0.40
21	0.2920	++	0.2982	-	0.3161	2.80	0.3184	0.00
28		bitter		bitter	*0.3596	3.33	0.3312	0.00
35	(Insufficient sample)				*	3.00		0.00

* Average two trials

Table 3. The oxidation-reduction potential and the development of oxidized flavor in unhomogenized and homogenized pasteurized milk after various storage periods. Spring milk, 1939.

Storage (Days)	Oxidation-reduction potentials and flavor when Spring milk was Unhomogenized Eh	Unhomogenized Flavor	Homogenized Eh	Homogenized Flavor	Unhomogenized Eh	Unhomogenized Flavor	Homogenized Eh	Homogenized Flavor
	Trial I				Trial II			
0	0.3318	-	0.3318	-	0.3230	-	0.3066	-
3	0.3586	?	0.3657	-	0.4386	-	0.4386	-
7	0.4066	+++	0.4406	-	0.3507	?	0.4376	-
10	0.4209	+++	0.4339	-	0.3757	?	0.3657	-
14	0.4300	+?	0.4280	-	0.4342	?	0.4462	-
21	0.4084	+++	0.3492	-	0.4137	+	0.4167	-
28	0.3517	+++	0.3102	bitter	0.4417	++	0.3215	-
35	0.3862	++	0.2222	bitter	0.4027	+++	0.3962	+
	Trial III				Trial IV			
0	0.3087	-	0.2952	-	0.3212	-	0.3254	-
3	0.3170	+	0.3204	-	0.3216	+++	0.3338	-
7	0.3348	++	0.3588	-	0.2836	+++	0.3462	++
10	0.3662	++	0.3898	-	0.3462	+++	0.3777	-
14	0.3806	+++	0.3922	-	0.3994	+++	0.4188	-
21	0.3757	+++	0.3908	bitter	0.3757	++	0.3606	bitter
28	0.3287	+++	0.3472	+	0.2957	+++	0.3762	stale +
35	0.2807	+++	0.3227	-	0.2777	+++	0.2374	sour
	Trial V				Average (5 trials)			
0	0.3236	-	0.3269	-	0.3217	0.00	0.3172	0.00
3	0.3182	-	0.3192	-	0.3508	1.40	0.3595	0.00
7	0.3432	-	0.3672	-	0.3438	2.40	0.3901	0.60
10	0.3528	++	0.3742	-	0.3724	3.00	0.3883	0.20
14	0.3792	++	0.3918	-	0.4047	2.80	0.4154	0.00
21	0.3892	+++	0.2862	bitter	0.3925	3.40	0.3607	0.00
28	0.3174	++	0.2514	bitter	0.3470	3.60	0.3212	1.00
35	Lost		Lost		*0.3368	4.00	0.2946	0.60

* Average of four trials.

Table 4. The oxidation-reduction potential and the development of oxidized flavor in unhomogenized and homogenized pasteurized milk after various storage periods. Spring milk, 1940.

Storage (Days)	Oxidation-reduction potentials and flavor when Spring milk was Unhomogenized: Eh	Unhomogenized: Flavor	Homogenized: Eh	Homogenized: Flavor	Unhomogenized: Eh	Unhomogenized: Flavor	Homogenized: Eh	Homogenized: Flavor
	Trial I				Trial II			
0	0.3289	-	0.3234	-	0.3490	-	0.3526	-
1	0.3205	-	0.3233	-	0.3261	-	0.3316	-
3	0.3297	-	0.3399	-	0.3233	+	0.3601	-
7	0.3160	+	0.3329	-	0.4396	+	0.4520	-
10	0.3686	+?	0.3796	-	lost		lost	
14	lost		lost		"		"	
21	"		"		"		"	
28	"		"		"		"	
	Trial III				Trial IV			
0	0.3254	-	0.3240	-	0.3127	-	0.2972	-
1	0.3425	-	0.3294	-	0.3198	-	0.3242	-
3	0.3523	-	0.3599	-	lost		lost	-
7	lost		lost		0.4165	?	0.4294	-
10	0.3965	+	0.4003	-	0.4002	++	0.3944	bitter
14	0.4428	+++	0.4403	-	0.3997	+	0.4054	-
21	0.3908	++	0.3785	-	0.3574	bitter	0.3454	bitter
28	0.3738	+	0.3705	-	0.2929	bitter	-0.0342	sour
	Average (4 trials)							
0	0.3294	0.00	0.3243	0.00				
1	0.3274	0.00	0.3271	0.00				
3	*0.3351	0.66	0.3533	0.00				
7	*0.3907	1.66	0.4048	0.00				
10	*0.3885	2.66	0.3914	0.00				
14	#0.4212	3.00	0.4228	0.00				
21	#0.3741	3.00	0.3620	0.00				
28	#0.3333	2.00	0.3705	0.00				

* Average of three trials.
\# Average of two trials.

Table 5. The oxidation-reduction potential and the development of oxidized flavor in unhomogenized and homogenized pasteurized milk after various storage periods. Average of all trials.

Storage (Days)	Oxidation-reduction potentials and flavor when milk was: Unhomogenized Eh	Unhomogenized Flavor	Homogenized Eh	Homogenized Flavor
	Average (all trials)			
0	0.3123	0.00	0.3044	0.00
1	0.3272	0.00	0.3271	0.00
3	0.3276	2.06	0.3431	0.00
7	0.3446	2.94	0.3649	0.28
10	0.3672	3.00	0.3666	0.22
14	0.4094	2.86	0.4175	0.00
17	0.3861	3.20	0.3535	0.00
21	0.3576	3.09	0.3433	0.00
28	0.3560	3.07	0.3501	0.29
35	0.3222	3.11	0.3017	0.22

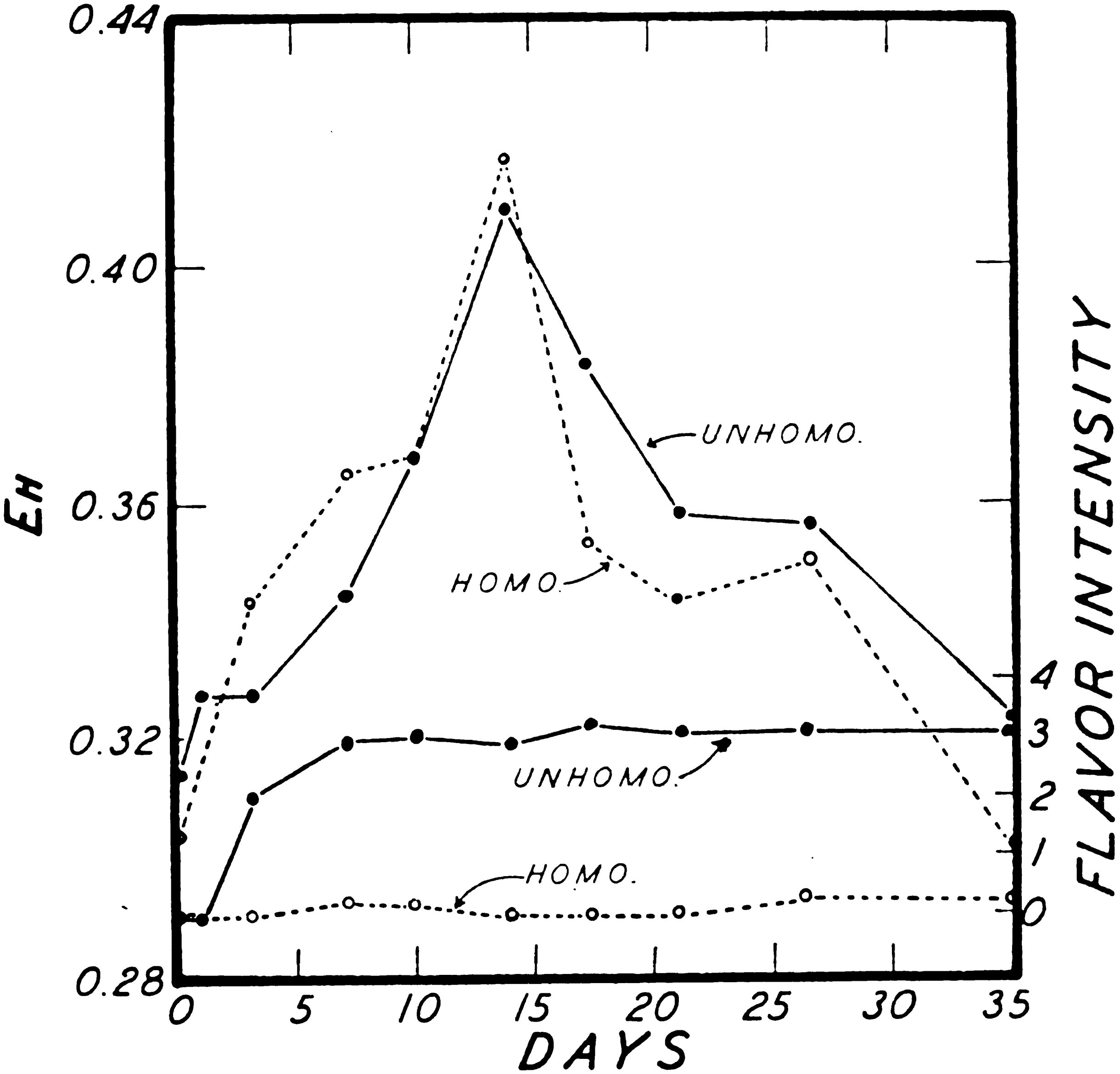

Figure 1. Relationship between oxidation-reduction potentials and the development of oxidized flavor in unhomogenized and homogenized milk over long storage periods.

was slightly less rapid in the unhomogenized than in the homogenized milk. However, no very significant differences in oxidation-reduction potential occurred between the unhomogenized and homogenized milk.

No oxidized flavor occurred in any of the samples after only one day of storage, but was usually quite pronounced by the third day of storage. On the third day of storage the unhomogenized milk merited an oxidized flavor ration of 2.06. After seven days of storage the rating increased to 2.94, remaining relatively constant until the seventeenth day of storage when it increased to 3.20 and stayed above 3.00 throughout the remainder of the holding period. The development of oxidized flavor in the unhomogenized milk was paralleled by an increase in the oxidation-reduction potential.

An oxidized flavor developed in only a very few cases in the homogenized milk even though the milk was stored as long as 35 days, showing the stabilizing effect of homogenization against oxidized flavor development over long storage periods. A rise in oxidation-reduction potential in the homogenized milk very similar to that observed in the unhomogenized milk, was not accompanied by the development of oxidized flavor. Results similar to these were secured in milk obtained at various periods throughout the year as shown by the various tables.

The summarized results of the oxidation-reduction potential and the development of oxidized flavor in unhomogenized and homogenized milk presented in table 5 are shown graphically in figure 1.

<u>The effect of copper upon the oxidation-reduction potential and upon the oxidized flavor in unhomogenized and homogenized milk over long storage periods.</u>

In this study the pasteurized milk was divided into three lots. Lot I was kept as a control while lots II and III were homogenized at 1,500 and 2,500 pounds pressure respectively. Each lot was then divided into three portions to which copper was added at the rate of 0, 1 and 3 p.p.m. respectively. Oxidation-reduction potential measurements and flavor determinations were made immediately after processing and after 1, 3, 7, 10, 14, 21 and 28 days of storage at 35° to 40° F. The experimental data are tabulated in tables 6, 7 and 8 with the average results of all trials summarized in table 9 and plotted on figures 2, 3 and 4.

An examination of the data shows that the addition of 1 p.p.m. of copper caused a rise in oxidation-reduction potential in both the unhomogenized and homogenized milk. The addition of 3 p.p.m. of copper caused a further increase but the rate of increase per part of copper added was not so great as when only 1 p.p.m. of copper was added. After 1 day of storage the potential was practically the same in the milk containing 1 p.p.m. of copper as it was in the milk containing 3 p.p.m. of added copper. The maximum potential of the copper treated milk usually occurred after one day of storage. After reaching the maximum the potential decreased, with the milk containing 3 p.p.m. of copper decreasing more rapidly and to a lower final potential than the milk containing only 1 p.p.m. of added copper. The unhomogenized milk treated with copper showed a slightly more rapid decrease in potential than the homogenized milk which was treated with copper.

The unhomogenized and homogenized milk to which no copper had been added showed a gradual rise in oxidation-reduction potential on storage. The maximum potential occurred on the fourteenth day of storage, after which there was a tendency for the potential to decrease. The maximum potential attained by the milk not contaminated with copper was practically the same as that attained in the copper treated milk. However, the day of storage at which the maximum potential was reached was greatly different, being reached the first day of storage in the latter but not until the fourteenth day of storage in the former.

An examination of the development of oxidized flavor shows that in the milk not treated with copper the oxidized flavor developed after 10, 21 and 28 days of storage in the unhomogenized milk and the milk homogenized at 1,500 and 2,500 pounds respectively. The addition of 1 p.p.m. of copper to the unhomogenized milk caused a slight oxidized flavor after one day of storage. The intensity of the flavor increased rapidly until the seventh day of storage, after which the intensity decreased a little, but gradually increased again until at the end of the storage period when a flavor rating of 4.0 was obtained. The addition of 3 p.p.m. of copper to the unhomogenized milk caused a strong oxidized flavor to develop quickly. After one week of storage the milk had an oxidized flavor rating of 4.0 and remained very strongly oxidized throughout the storage period.

Homogenization at 1,500 and 2,500 pounds pressure delayed the development of an oxidized flavor in milk to which copper had been added. The higher pressure was more effective in retarding the flavor than the lower pressure. The addition of 1 p.p.m. of copper to the homogenized milk did not cause as intense an oxidized flavor as did the addition of 3 p.p.m.

Table 6. The effect of copper upon the oxidation-reduction potential and upon the development of oxidized flavor in unhomogenized and homogenized pasteurized milk after various storage periods at a low temperature. (Trial 1).

Storage (Days)	Copper added (p.p.m.)	Oxidation-reduction potentials and flavor when milk was homogenized at various pressures: 0# Eh	0# Flavor	1500# Eh	1500# Flavor	2500# Eh	2500# Flavor
0	0	0.3300	-	0.3300	-	0.3260	-
	1	0.3540	-	0.3590	-	0.3850	-
	3	0.3888	-	0.3998	-	0.4038	-
1	0	0.3220	-	0.3183	-	0.3186	-
	1	0.3819	?	0.3909	+	0.3929	-
	3	0.3939	-	0.3912	-	0.3954	-
3	0	0.3099	-	0.3137	-	0.3320	-
	1	0.3749	-	0.3796	+	0.3809	-
	3	0.3849	+	0.3852	-	0.3992	-
7	0	0.3334	-	0.3412	-	0.3832	-
	1	0.3908	+	0.3982	-	0.3982	-
	3	0.3892	+++	0.3992	+	0.4002	+
10	0	0.3372	-	0.3778	-	0.3884	-
	1	0.3964	?	0.3984	-	0.3982	-
	3	0.3612	+++	0.3892	+?	0.3892	+
14	0	0.3500	-	0.3930	-	0.3980	-
	1	0.3996	?	0.4070	-	0.4080	-
	3	0.3994	+++	0.3928	++	0.4008	-
21	0	0.3366	+	0.3612	-	0.3669	-
	1	0.3004	+	0.3654	?	0.3590	+
	3	0.3160	+++	0.3504	+++	0.3540	++
28	0	0.3852	+?	0.3666	?	0.3480	bitter
	1	0.3476	+++	0.3860	++	0.3442	sour
	3	0.2792	+++	0.3317	+++	0.3577	+++

Table 7. The effect of copper upon the oxidation-reduction potential and the development of oxidized flavor in unhomogenized and homogenized pasteurized milk after various storage periods at a low temperature. (Trial 2).

Storage (Days)	Copper added (p.p.m.)	Oxidation-reduction potentials and flavor when milk was homogenized at various pressures: 0# Eh	0# Flavor	1500# Eh	1500# Flavor	2500# Eh	2500# Flavor
0	0	0.3178	-	0.3102	-	0.3098	-
	1	0.3697	-	0.3757	-	0.3807	-
	3	0.3927	-	0.3887	-	0.3957	?
1	0	0.3514	-	0.3606	-	0.3526	-
	1	0.4016	-	0.4038	-	0.4028	-
	3	0.4068	+++	0.4078	-	0.4058	-
3	0	0.3552	-	0.3802	-	0.3812	-
	1	0.4012	++	0.4082	?	0.4036	-
	3	0.4169	+++	0.4156	++	0.4166	+
7	0	0.3542	-	0.3684	-	0.3794	-
	1	0.3764	++	0.3822	-	0.3787	-
	3	0.3647	+++	0.3777	+	0.3822	-
10	0	0.3888	-	0.3878	-	0.3932	-
	1	0.3882	++	0.3967	?	0.3977	-
	3	0.3612	+++	0.3882	+++	0.3897	+
14	0	0.4370	++	0.4032	-	0.4082	-
	1	0.3898	+++	0.4062	-	0.4002	++
	3	0.3747	+++	0.3767	++	0.3847	+
21	0	0.4069	++	0.2904	-	0.3364	-
	1	0.3254	+++	0.3298	+++	0.3648	++
	3	0.3078	+++	0.3088	++	0.2908	++
28	0	0.3570	++	0.3397	++	0.3237	++
	1	0.3359	+++	-0.1031	sour	0.2594	+
	3	0.2724	+++	0.2566	+++	0.2446	++

Table 8. <u>The effect of copper upon the oxidation-reduction potential and upon the development of oxidized flavor in unhomogenized and homogenized pasteurized milk after various storage periods at a low temperature. (Trial 3).</u>

Storage (Days)	Copper added (p.p.m.)	Oxidation-reduction potentials and flavor when milk was homogenized at various pressures: 0 — Eh	0 — Flavor	2500 — Eh	2500 — Flavor
0	0	0.3137	-	0.3327	-
	1	0.3807	-	0.3587	-
	3	0.3757	-	0.3602	-
1	0	0.3362	-	0.3697	-
	1	0.4637	-	0.4567	-
	3	0.4467	++ ?	0.4437	?
3	0	0.3701	-	0.3996	-
	1	0.4251	++	0.4531	?
	3	0.4391	+++	0.4271	+?
7	0	0.3677	-	0.3377	-
	1	0.3957	+++	0.4367	-
	3	0.3902	+++	0.4382	+++
10	0	0.3464	?	0.3578	-
	1	0.3324	+++	0.3642	+?
	3	0.2987	+++	0.3197	++
14	0	0.4336	-	0.4069	-
	1	0.3989	++?	0.4189	-
	3	0.3070	+++	0.3166	++?

Table 9. The effect of copper upon the oxidation-reduction potential and upon the development of oxidized flavor in unhomogenized and homogenized pasteurized milk after various storage periods at a low temperature. (Average of all trials.)

Storage (Days)	Copper added (p.p.m.)	Oxidation-reduction potentials and flavor when milk was homogenized at various pressures					
		0		1500		2500	
		Eh	Flavor	Eh	Flavor	Eh	Flavor
0	0	0.3205	0.00	0.3201	0.00	0.3228	0.00
	1	0.3681	0.00	0.3673	0.00	0.3751	0.00
	3	0.3857	0.00	0.3942	0.00	0.3866	0.33
1	0	0.3365	0.00	0.3394	0.00	0.3470	0.00
	1	0.4157	0.67	0.3973	1.00	0.4175	0.00
	3	0.4158	2.33	0.3995	0.00	0.4150	0.33
3	0	0.3451	0.00	0.3469	0.00	0.3709	0.00
	1	0.4004	2.00	0.3939	1.50	0.4125	0.33
	3	0.4136	3.33	0.4004	1.50	0.4143	1.33
7	0	0.3518	0.00	0.3548	0.00	0.3668	0.00
	1	0.3876	3.00	0.3902	0.00	0.4045	0.00
	3	0.3814	4.00	0.3685	2.00	0.4069	2.00
10	0	0.3575	0.33	0.3828	0.00	0.3798	0.00
	1	0.3723	2.67	0.3976	0.50	0.3867	0.67
	3	0.3404	4.00	0.3887	3.00	0.3662	2.33
14	0	0.4069	1.00	0.3981	0.00	0.4044	0.00
	1	0.3961	2.33	0.4066	0.00	0.4090	1.00
	3	0.3604	4.00	0.3848	3.00	0.3674	1.67
21	0	0.3718	2.50	0.3258	2.00	0.3517	0.00
	1	0.3129	3.00	0.3476	2.50	0.3619	2.50
	3	0.3119	4.00	0.3296	3.50	0.3224	3.00
28	0	0.3711	2.50	0.3532	2.00	0.3659	1.50
	1	0.3418	4.00	0.3660	3.00	0.3018	2.00
	3	0.2758	4.00	0.2942	4.00	0.3011	3.50

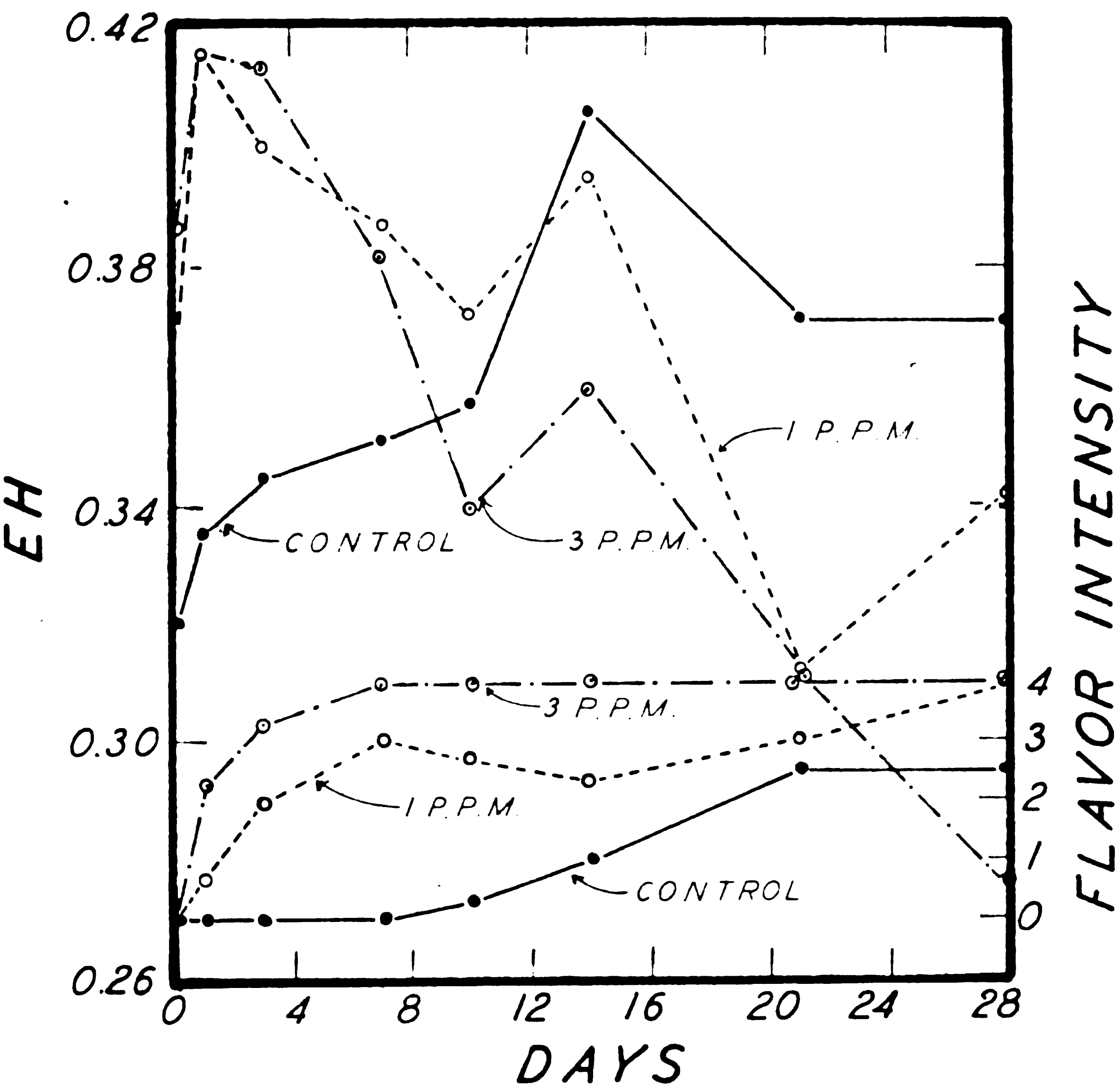

Figure 2. Effect of copper upon the oxidation-reduction potential and upon the development of oxidized flavor in unhomogenized milk.

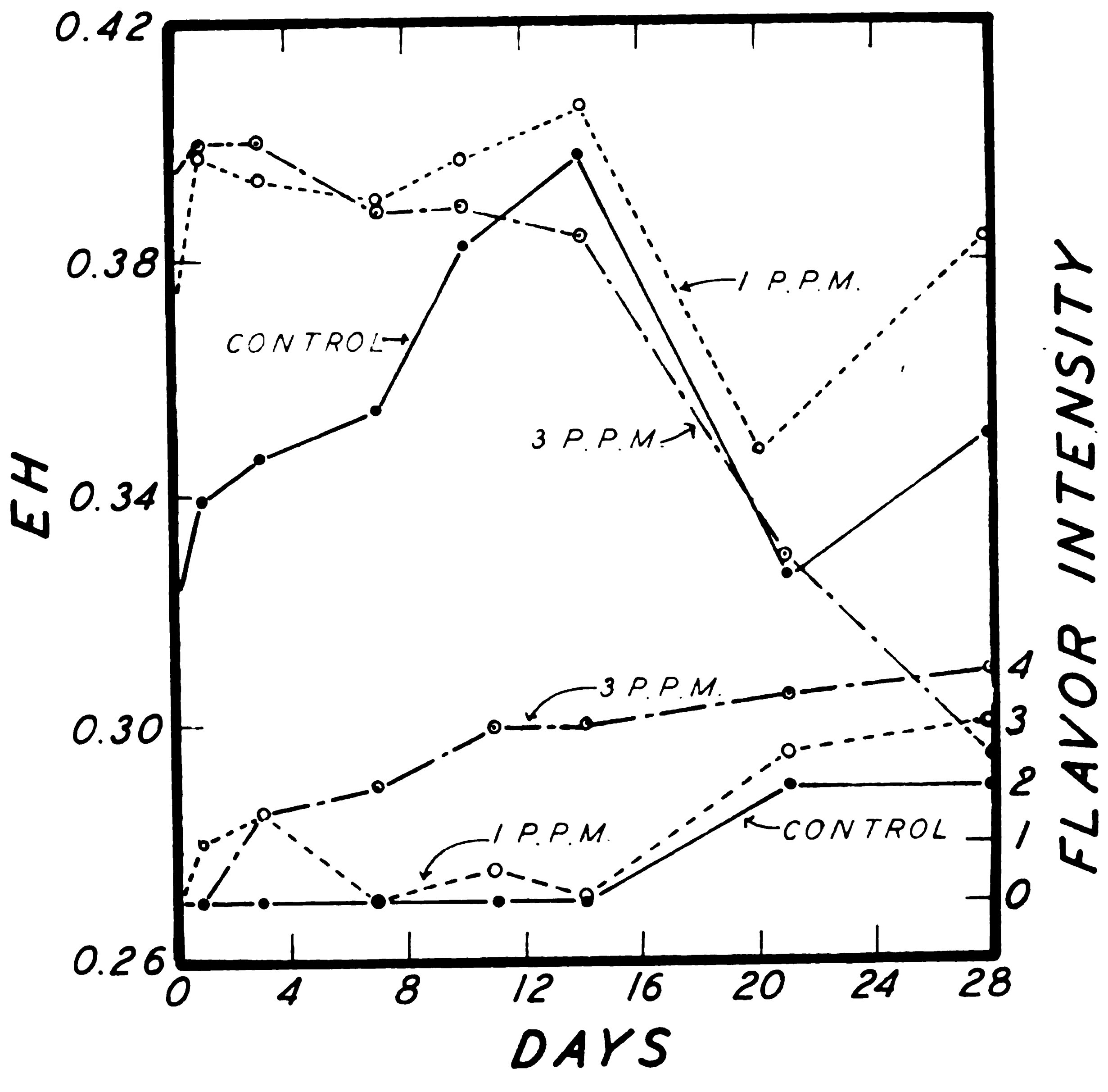

Figure 3. Effect of copper upon the oxidation-reduction potential and upon the development of oxidized flavor in milk homogenized at 1,500 pounds pressure.

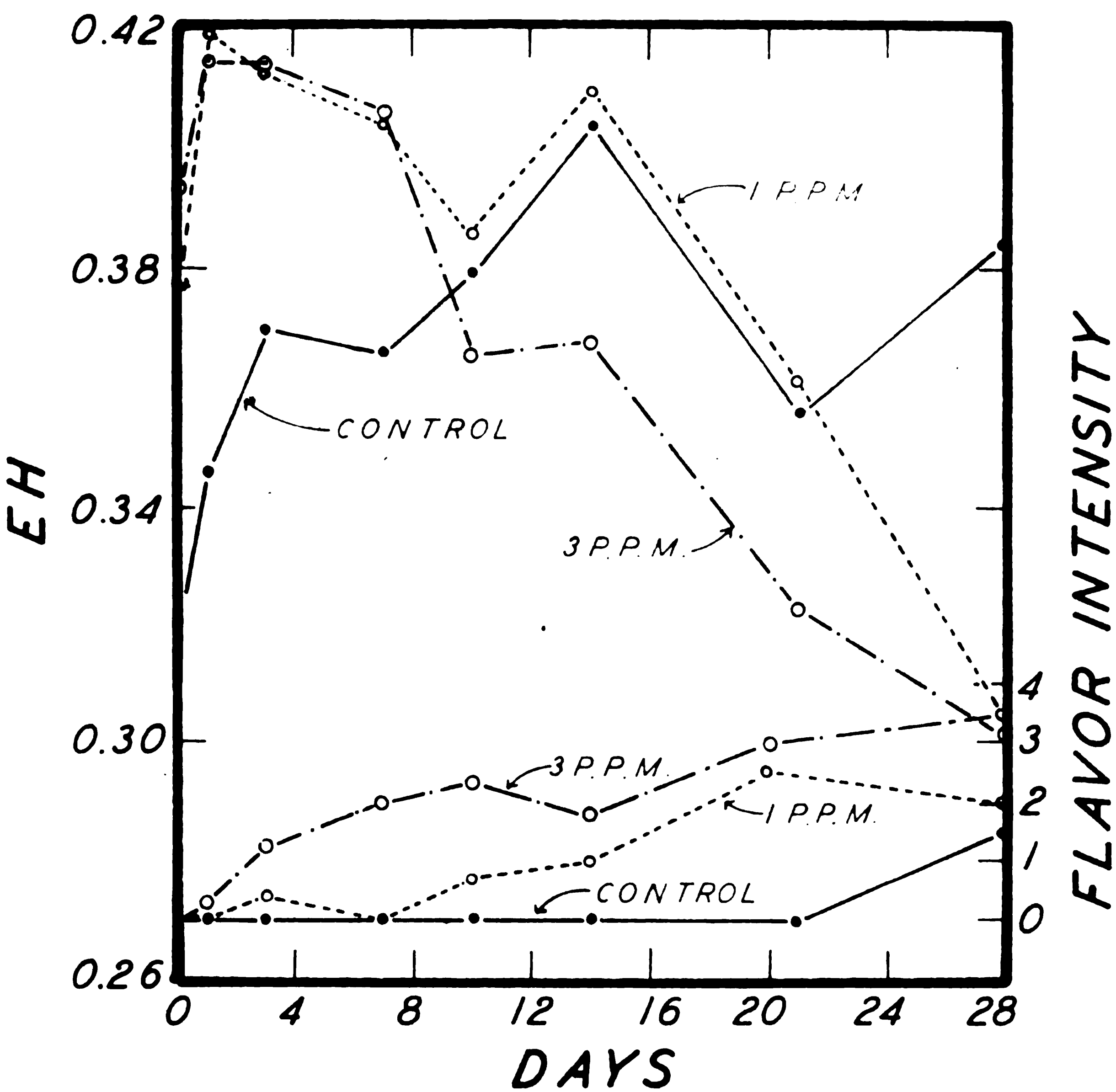

Figure 4. Effect of copper upon the oxidation-reduction potential and upon the development of oxidized flavor in milk homogenized at 2,500 pounds pressure.

of copper. The rate at which the oxidized flavor increased in intensity was more gradual in the homogenized than in the unhomogenized. However, the oxidized flavor in the homogenized milk containing 3 p.p.m. of added copper was nearly as intense at the end of the storage period as it was in the unhomogenized milk to which copper had been added.

An increase in oxidation-reduction potential in the unhomogenized, copper-free milk was accompanied by a delayed development of oxidized flavor, whereas, the increase in the potential of the copper treated milk was closely paralleled by the development of oxidized flavor.

An increase in oxidation-reduction potential in homogenized milk not treated with copper was not paralleled by the development of an oxidized flavor. There was some parallelism, however, between an increase in oxidation-reduction potential and the development of oxidized flavor in homogenized milk treated with copper.

<u>The effect of removing the fat globule membrane by churning and washing upon the development of oxidized flavor in the remade homogenized milk.</u>

In an effort to show the effect of removing the fat globule membrane upon the development of oxidized flavor in milk, flavor studies were made on unhomogenized milk, homogenized milk and remade homogenized milk in which the fat had been washed 1, 2 and 3 times as outlined in the procedure. Copper was added to the samples at the rate of 0.0, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.5 p.p.m. and were studied organoleptically after 1, 3 and 7 days of storage at 35° to 40° F. The data are presented in table 10, with the average results of all trials presented in table 11.

An examination of the data shows considerable variations in the susceptibility of different milks to the development of oxidized flavor. The milk in trials 1, 2 and 3, which were run during March and April, were quite susceptible to oxidized flavor development, whereas, trials 4 and 5, which were run during June when the cows were on pasture, was very resistant to the development of oxidized flavor.

Oxidized flavor developed more rapidly in the unhomogenized samples than it did in the unwashed homogenized or in the washed homogenized samples. The unwashed homogenized samples were more resistant against oxidized flavor development than the control or the washed samples. The greater susceptibility toward oxidized flavor of the washed samples than the unwashed homogenized samples may have been due to the severe treatment, such as separation, churning, washing and homogenization to which the fat was subjected during processing.

The samples which were washed once and twice were a little more susceptible to off flavor development than the samples which were washed three times. This seems to be in keeping with the theory that lecithin is the substance which becomes oxidized and causes the oxidized flavor, the removal of which from the milk results in greater stability against oxidized flavor.

The addition of copper increased the intensity of the oxidized flavor that developed in the unhomogenized and the washed homogenized samples. An astringent or puckery flavor was often encountered in the remade milk especially in those samples containing the higher concentrations of copper.

The lecithin content of the buttermilk from each of the three churnings was determined in order to see what relationship existed between the amount of lecithin removed by churning and washing and the development of oxidized flavor.

An examination of the data shows that the buttermilk from the first churning contained the largest percentage of lecithin. The buttermilk from the second churning contained less lecithin then the buttermilk from the first churning, but more than the buttermilk from the third churning, showing that each successive churning and washing lowered the amount of lecithin that remained in the milk. The lecithin contents of the buttermilk from the first, second and third churnings were 0.1663, 0.1160 and 0.0914 per cent respectively. The percentage of lecithin in the fat of the buttermilk varied considerably because of large variation in the fat content of the buttermilk. The average percentages of lecithin in the fat of the buttermilk from the first, second and third churnings were 2.5308, 1.2195 and 0.7337 per cent respectively, again showing a decrease in the lecithin content in the milk due to churning and washing.

Table 10. The effect of removing the fat globule membrane by churning and washing upon the development of oxidized flavor in the remade homogenized milk after storage for various periods at a low temperature.

Copper added	The oxidized flavor development in milk subjected to homogenization and washing when stored for 1, 3 and 7 days.														
	Unhomogenized			Homogenized (2500#)											
	Control			Unwashed			Washed once			Washed Twice			Washed three times		
(p.p.m.)	1 day	3 days	7 days	1 day	3 days	7 days	1 day	3 days	7 days	1 day	3 days	7 days	1 day	3 days	7 days
Trial I (March 28, 1939)															
0.0	-	-	++	-	-	-	-	-	-	-	+++	-	-	-	-
0.2	+++	+++	+++	-	-	-	++	+	?	++	+++	-	-	-	-
0.4	+++	+++	+++	-	-	-	++	+++	+++	++	+++	-	-	-	-
0.6	+++	+++	+++	-	-	+	++	+++	?	-	+++	-	-	-	-
0.8	+++	+++	+++	-	-	++	+	++	+++	-	+++	+++	-	-	-
1.0	+++	+++	+++	-	-	++	+	+++	+++	+++	++	+++	-	-	-
1.5	++	+++	+++	-	-	+++	+	+++	+++	++	+++	++	-	++	-
Trial II (April 1, 1939)															
0.0	-	-	++	-	-	-	-	-	-	-	-	-	-	-	-
0.2	+++	+++	+++	-	-	-	?	-	-	++	-	-	+	-	-
0.4	+++	+++	+++	-	-	-	+	-	-	++	-	?	-	-	-
0.5	+++	+++	+++	-	-	-	-	-	++	+?	-	-	-	-	-
0.8	+++	+++	+++	-	-	-	-	-	++	+?	-	-	-	-	-
1.0	+++	+++	+++	-	-	++	-	-	+++	++	?	?	-	-	-
1.5	+++	+++	+++	-	-	+++	++	+++	+++	++	++	+++	?	++	++

Table 10. (Continued)

Trial III (April 16, 1939)

0.0	-	-	-	-	-	-	-	?	-	-	?	-	-	-	-
0.2	+++	+++	+++	-	-	-	-	?	-	++	?	-	+	-	-
0.4	+++	+++	+++	-	-	-	-	+	-	+	?	-	+	-	-
0.6	+++	+++	+++	-	-	-	-	?	-	-	-	-	+	-	-
0.8	+++	+++	+++	-	-	-	-	+	-	++	-	-	+	-	-
1.0	+++	+++	+++	-	-	-	-	+	-	?	?	-	+	+	-
1.5	+++	+++	+++	-	-	-	-	+	-	?	?	++	+	+	-

Trial IV (June 12, 1939)

0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.4	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
0.6	-	-	++	-	-	-	-	-	-	-	-	-	-	-	-
0.8	-	+	++	-	-	-	-	-	-	-	-	-	-	-	-
1.0	-	+	++	-	-	-	-	-	-	-	-	-	-	-	-
1.5	-	+++	+++	-	-	-	-	-	-	-	-	-	-	-	-

Trial V (June 29, 1939)

0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.8	-	-	+	-	-	-	-	-	-	-	-	-	?	-	-
1.0	-	+	+	-	-	-	-	-	-	-	-	-	+	+	-
1.5	-	+	+++	-	-	-	-	-	-	-	-	-	-	-	-

Table 11. The effect of removing the fat globule membrane by churning and washing upon the development of oxidized flavor in the remade homogenized milk after storage for various periods at a low temperature. (Average of all trials)

Copper added (p.p.m.)	The development of oxidized flavor in milk subjected to homogenization and washing when stored for 1, 3 and 7 days.														
	Unhomogenized			Homogenized (2500#)											
	Control			Unwashed			Washed once			Washed twice			Washed three times		
	1 day	3 days	7 days	1 day	3 days	7 days	1 day	3 days	7 days	1 day	3 days	7 days	1 day	3 days	7 days
0.0	0.0	0.0	1.2	0.0	0.0	0.0	0.0	0.2	0.0	0.0	1.0	0.0	0.0	0.0	0.0
0.2	2.4	2.4	2.4	0.0	0.0	0.0	0.8	0.6	0.2	0.8	1.0	0.0	0.8	0.0	0.0
0.4	2.4	2.4	2.8	0.0	0.0	0.0	1.0	1.2	0.8	1.6	1.0	0.2	0.4	0.0	0.0
0.6	2.4	2.4	3.0	0.0	0.0	0.4	0.6	1.0	0.8	0.4	0.8	0.0	0.4	0.0	0.0
0.8	2.4	2.8	3.4	0.0	0.0	0.6	0.4	1.0	1.4	1.0	0.8	0.8	0.8	0.0	0.0
1.0	2.4	3.2	3.4	0.0	0.0	1.2	0.4	1.2	1.6	1.6	0.1	1.0	0.8	0.4	0.2
1.5	2.2	3.6	4.0	0.0	0.0	1.4	1.0	2.0	1.8	1.4	1.6	2.0	0.6	1.6	0.6

Table 12. The lecithin content of the resulting buttermilk when fat washed one, two, and three times was churned.

Churning (no.)	Analysis of buttermilk for lecithin						
		Fat				Lecithin	
	Buttermilk (Gms.)	(Gms.)	Per cent	Phosphorus (Mg.)	Lecithin (Mg.)	in buttermilk %	in fat %
Trial I							
1	9.8386	1.0241	10.4090	0.6431	16.6820	0.1696	1.6289
2	10.3219	1.1282	10.9301	0.4249	11.0167	0.1067	0.9765
3	10.0837	1.1952	11:8528	0.3205	8.3138	0.0824	0.6956
Trial II							
1	9.9794	0.5258	5.2689	0.6211	16.1113	0.1614	3.0641
2	9.9389	1.0224	10.2869	0.4664	12.0984	0.1217	1.1833
3	9.9019	1.2590	12.7147	0.3847	9.9791	0.1008	0.7926
Trial III							
1	10.1841	0.4218	4.1418	0.6593	17.1022	0.1679	4.0546
2	10.1760	0.7447	7.3182	0.4700	12.1918	0.1198	1.6371
3	9.8724	1.2783	12.8184	0.3505	9.0920	0.0912	0.7113
Average of all trials							
1	10.0007	0.6572	6.5715	0.6412	16.6337	0.1663	2.5308
2	10.1456	0.9651	9.5125	0.4537	11.7690	0.1160	1.2195
3	9.9860	1.2442	12.4620	0.3519	9.1283	0.0914	0.7337

The data obtained regarding the lecithin content of the buttermilk are presented in table 12.

<u>The development of oxidized flavor in milk made from unhomogenized cream and skimmilk and homogenized cream and skim milk after storage for various period at a low temperature.</u>

In an effort to determine whether the substance or substances in milk, which are affected by homogenization in such a manner that oxidized flavor development is retarded or prevented, are associated with the fat or with the serum fraction, flavor studies were made upon unhomogenized milk, homogenized milk and milk reconstituted from unhomogenized cream plus unhomogenized skim milk, unhomogenized cream plus homogenized skim milk, homogenized cream plus unhomogenized skim milk and homogenized cream plus homogenized skim milk. The flavor was studied organoleptically after 0, 1, 3, 7, 10, and 14 days of storage at 35° to 40° F. The data obtained are presented in table 13 with the average results of all trials presented in table 14.

An examination of trial 1 shows that the milk was very resistent to oxidized flavor development. Copper was added to the milk in trials 2, 3, 4 and 5 in order to induce the development of oxidized flavor.

The unhomogenized milk was the most susceptible to the development of oxidized flavor. The milk reconstituted from unhomogenized cream and unhomogenized milk was nearly as susceptible to the development of oxidized flavor as was the unhomogenized milk. The addition of unhomogenized cream to homogenized skim milk resulted in a milk which was slightly more resistant to the development of oxidized flavor than the milk resulting from the mixing of unhomogenized cream and skim milk. When the milk was reconstituted from homogenized cream and unhomogenized skim milk a further increase in flavor stability was noted. The flavor stability was still

Table 13. The development of oxidized flavor in milk reconstituted from unhomogenized cream and skim milk and homogenized cream and skim milk after storage for various periods at a low temperature.

Storage (Days)	Oxidized flavor development when milk was: Unhomogenized	Unhomogenized cream plus Unhomogenized skim milk	Unhomogenized cream plus Homogenized skim milk	Homogenized cream plus Unhomogenized skim milk	Homogenized cream plus Homogenized skim milk	Homogenized
Trial I						
0	-	-	-	-	-	-
1	-	-	-	-	-	-
3	-	-	-	-	-	-
7	-	?	-	-	-	-
10	-	+	bitter	+	-	-
14	bitter	-	bitter	-	bitter	-
Trial II						
0	-	-	-	-	-	-
1	+	-	-	-	-	-
3	++	-	-	-	-	-
7	++	-	-	-	-	-
10	+++	-	-	-	-	-
14	+++	-	-	-	-	-
Trial III						
0	-	-	-	-	-	-
1	?	+	+	-	-	-
3	++	++	++	+	+	?
7	++	++	++	+	++	-
10	+++	++	++	++	+	-
14	+++	++	bitter	bitter	bitter	-
Trial IV						
0	-	-	-	-	-	-
1	-	?	+	-	-	-
3	+++	+	++	-	-	-
7	++	+++	++	++	-	-
10	++	+++	++	++	-	-
14	++	+++	+++	++	-	-
Trial V						
0	-	-	-	-	-	-
1	-	-	-	-	-	-
3	++	?	?	+	±	-
7	+++	++	+	+	-	-
10	+++	++	++	+	?	-
14	+++	+++	++	++	+	-

Table 14. The development of oxidized flavor in milk reconstituted from unhomogenized cream and skim milk and homogenized cream and skim milk after storage for various periods at a low temperature. (Average of all trials)

Storage (Days)	Oxidized flavor development when milk was					
	Unhomogenized	Unhomogenized cream plus		Homogenized cream plus		Homogenized
		Unhomogenized skim milk	Homogenized skim milk	Unhomogenized skim milk	Homogenized skim milk	
0	0.00	0.00	0.00	0.00	0.00	0.00
1	0.60	0.60	0.80	0.00	0.00	0.00
3	2.60	1.20	1.40	0.80	0.40	0.20
7	2.60	2.20	1.60	1.40	0.60	0.00
10	3.00	2.40	2.25	2.00	0.60	0.00
14	4.00	3.20	2.33	1.66	0.66	0.00
Total	12.80	9.60	7.58	5.86	2.26	0.20

greater in the milk reconstituted from homogenized cream and homogenized skim milk, but was not as resistant toward the development of oxidized flavor as the homogenized milk.

The data indicate that the substance or substances affected by homogenization in such a manner that oxidized flavor development is retarded, are more closely associated with the cream than with the skim milk. However, when only the skim milk was homogenized an increase in flavor stability was noted probably indicating that there are some substance or substances in the skim milk which are also affected by homogenization in such a manner as to increase flavor stability.

In order to show the comparative ability of the different milks to resist oxidized flavor development the average flavor rating of each milk for each day the samples were studied for oxidized flavor were totaled. The total flavor rating for the unhomogenized milk, and the milks made from unhomogenized cream plus unhomogenized skim milk, unhomogenized cream plus homogenized skim milk, homogenized cream plus unhomogenized skim milk, and homogenized cream plus homogenized skim milk, and homogenized milk were 12.80, 9.60, 7.58, 5.86, 2.26 and 0.20 respectively.

<u>The ascorbic acid content and the development of oxidized flavor in raw, unhomogenized pasteurized and homogenized pasteurized copper-free and copper-treated milk after various storage periods at a low temperature.</u>

In this study the milk was obtained from the college herd immediately after milking. Care was taken in order to prevent copper contamination. A portion of the raw milk was saved and the remainder was pasteurized at 143° F. for 30 minutes. The pasteurized milk was divided into two lots, one lot of which was homogenized at pasteurization temperature at a pressure of 2,500 pounds. The raw milk, unhomogenized pasteurized, and homogenized pasteurized milk were then divided into two portions and copper was added to one of the portions at the rate of 0.5 p.p.m. The samples were studied organoleptically for oxidized flavor and titrated for ascorbic acid content after storage for 0, 1, 2 and 7 days at 35° to 40° F. The data obtained are presented in tables 15 and 16.

An examination of the data presented in table 15 shows that the fresh copper-free milk contains slightly more ascorbic acid when raw than after pasteurization. Homogenization usually caused a further small decrease of ascorbic acid in the fresh pasteurized milk. The ascorbic acid disappeared more rapidly in the raw milk when stored than in the pasteurized milk, but not so rapidly as in the homogenized milk. The average ascorbic acid contents of the raw, unhomogenized pasteurized and homogenized pasteurized milk when fresh were 21.33, 20.47 and 20.19 milligrams per liter respectively, decreasing to 5.12, 8.19 and 3.53 milligrams of ascorbic acid per liter respectively after seven days of storage.

There was no development of oxidized flavor in any of the copper-free samples of milk after seven days of storage even though in some cases less than 2.0 milligrams of ascorbic acid remained in the samples at the end of the storage period.

An examination of the data presented in table 16 shows that pasteurization and homogenization decreased the ascorbic acid content of the fresh copper-treated milk to about the same degree as was noted in the copper-free samples. However, the ascorbic acid values for the fresh copper-treated milk were lower than those of the copper-free milk, which was probably due to the destructive effect of copper upon the ascorbic acid between the time of copper contamination and the titration for ascorbic acid content. The ascorbic acid disappeared quite rapidly on storage, having practically disappeared after one day of storage. Only a trace of ascorbic acid remained in the samples after two days of storage. The average ascorbic acid contents of the copper-free samples of raw, unhomogenized pasteurized and homogenized pasteurized milk after one day of storage were 19.25, 19.30 and 18.22 milligrams per liter respectively; whereas, the ascorbic acid contents of the copper-treated samples after one day of storage were 2.09, 4.18 and 4.17 milligrams per liter respectively.

Pasteurization seemed to retard to some extent the destruction of ascorbic acid in both the copper-free and copper-treated samples. Homogenization did not have much effect upon the rate of disappearance of ascorbic acid in the copper-treated milk during storage.

The disappearance of ascorbic acid in copper-free milk was not accompanied by the development of oxidized flavor. Some correlation existed between the development of oxidized flavor in copper-treated raw milk and the disappearance of ascorbic acid. The disappearance of ascorbic acid

Table 15. The ascorbic acid content and the development of oxidized flavor in raw, unhomogenized pasteurized and homogenized pasteurized milk after various storage periods at a low temperature.

Storage (days)	Ascorbic acid and oxidized flavor development when milk was: Raw — Ascorbic acid (Mg/L)	Raw — Flavor	Pasteurized, Unhomogenized — Ascorbic acid (Mg/L)	Pasteurized, Unhomogenized — Flavor	Pasteurized, Homogenized — Ascorbic acid (Mg/L)	Pasteurized, Homogenized — Flavor
Trial I December 27, 1939						
0	24.71	-	23.62	-	23.19	-
1	23.35	-	23.62	-	19.93	-
2	18.79	-	20.96	-	12.79	-
7	7.94	-	8.30	-	6.23	-
Trial II January 12, 1940						
0	22.87	-	21.56	-	21.93	-
1	21.42	-	21.56	-	19.97	-
2	19.60	-	19.75	-	19.60	-
7	lost		lost		lost	
Trial III January 24, 1940						
0	18.95	-	17.28	-	16.77	-
1	16.29	-	16.65	-	15.20	-
2	12.98	-	14.20	-	8.29	-
7	3.24	-	8.63	-	1.08	-
Trial IV April 1, 1940						
0	22.45	-	22.30	-	21.92	-
1	21.39	-	21.17	-	21.17	-
2	17.54	-	19.66	-	18.14	-
7	6.86	-	16.97	-	5.42	-
Trial V April 2, 1940						
0	19.88	-	19.66	-	19.28	-
1	16.25	-	17.77	-	15.35	-
2	13.00	-	14.58	-	12.42	-
7	5.78	-	4.91	-	2.53	-
Trial VI April 9, 1940						
0	19.13	-	18.41	-	18.05	-
1	16.82	-	18.05	-	17.69	-
2	16.03	-	15.16	-	14.01	-
7	1.80	-	2.15	-	2.37	-
Average of all trials						
0	21.33	0.00	20.47	0.00	20.19	0.00
1	19.25	0.00	19.80	0.00	18.22	0.00
2	16.32	0.00	17.39	0.00	14.21	0.00
7	5.12	0.00	8.19	0.00	3.53	0.00

Table 16. The effect of copper on the ascorbic acid content and the development of oxidized flavor in raw, unhomogenized pasteurized and homogenized pasteurized milk after various storage periods at a low temperature.

Storage (days)	Ascorbic acid content and oxidized flavor development when copper-treated milk was					
			Pasteurized			
	Raw		Unhomogenized		Homogenized	
	Ascorbic acid: (Mg/L)	Flavor	Ascorbic acid: (Mg/L)	Flavor	Ascorbic acid: (Mg/L)	Flavor
Trial I	December 27, 1939					
0	24.16	-	21.99	-	22.64	-
1	7.06	+++	7.87	+++	9.50	?
2	1.04	+++	0.78	+++	0.26	-
7	0.00	+++	0.26	+++	0.00	?
Trial II	January 12, 1940					
0	21.05	-	21.56	-	18.88	-
1	0.73	-	12.34	-	7.99	?
2	0.36	+++	5.08	++	1.45	-
7	lost		lost		lost	
Trial III	January 24, 1940					
0	18.00	-	16.91	-	16.18	-
1	0.00	-	0.72	++	1.09	-
2	0.00	-	0.00	+?	0.36	-
7	0.00	-	0.00	++	0.00	-
Trial IV	April 1, 1940					
0	22.30	-	22.68	-	21.02	-
1	3.63	-	3.02	-	4.38	-
2	0.76	?	0.00	++	0.00	+
7	0.00	-	0.00	+++	0.22	+
Trial V	April 2, 1940					
0	19.81	-	18.52	-	18.14	-
1	0.76	-	0.38	+	0.60	-
2	0.00	+	0.00	+++	0.00	-
7	0.00	sour	0.00	+++	0.00	-
Trial VI	April 9, 1940					
0	18.99	-	19.49	-	18.99	-
1	0.36	-	0.72	++	1.44	?
2	0.00	?	0.00	+++	0.00	-
7	0.36	++	0.22	+++	0.36	-
Average of all trials						
0	20.72	0.00	20.19	0.00	19.31	0.00
1	2.01	0.67	4.18	2.00	4.17	0.50
2	0.36	1.67	0.98	2.33	0.35	0.33
7	0.07	1.40	0.10	3.80	0.12	0.60

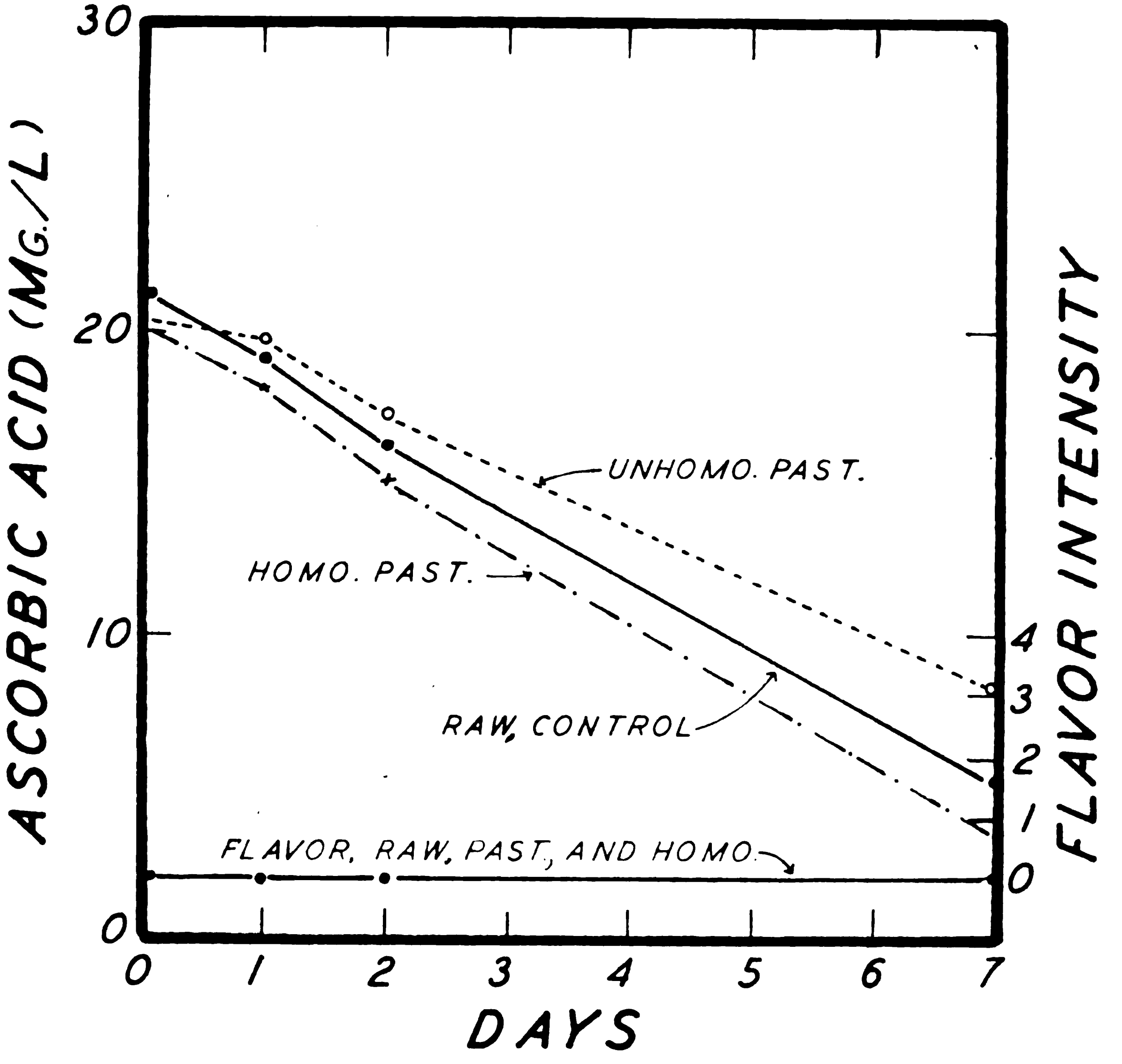

Figure 5. The ascorbic acid content and the development of oxidized flavor in raw, unhomogenized pasteurized and homogenized pasteurized milk after various storage periods.

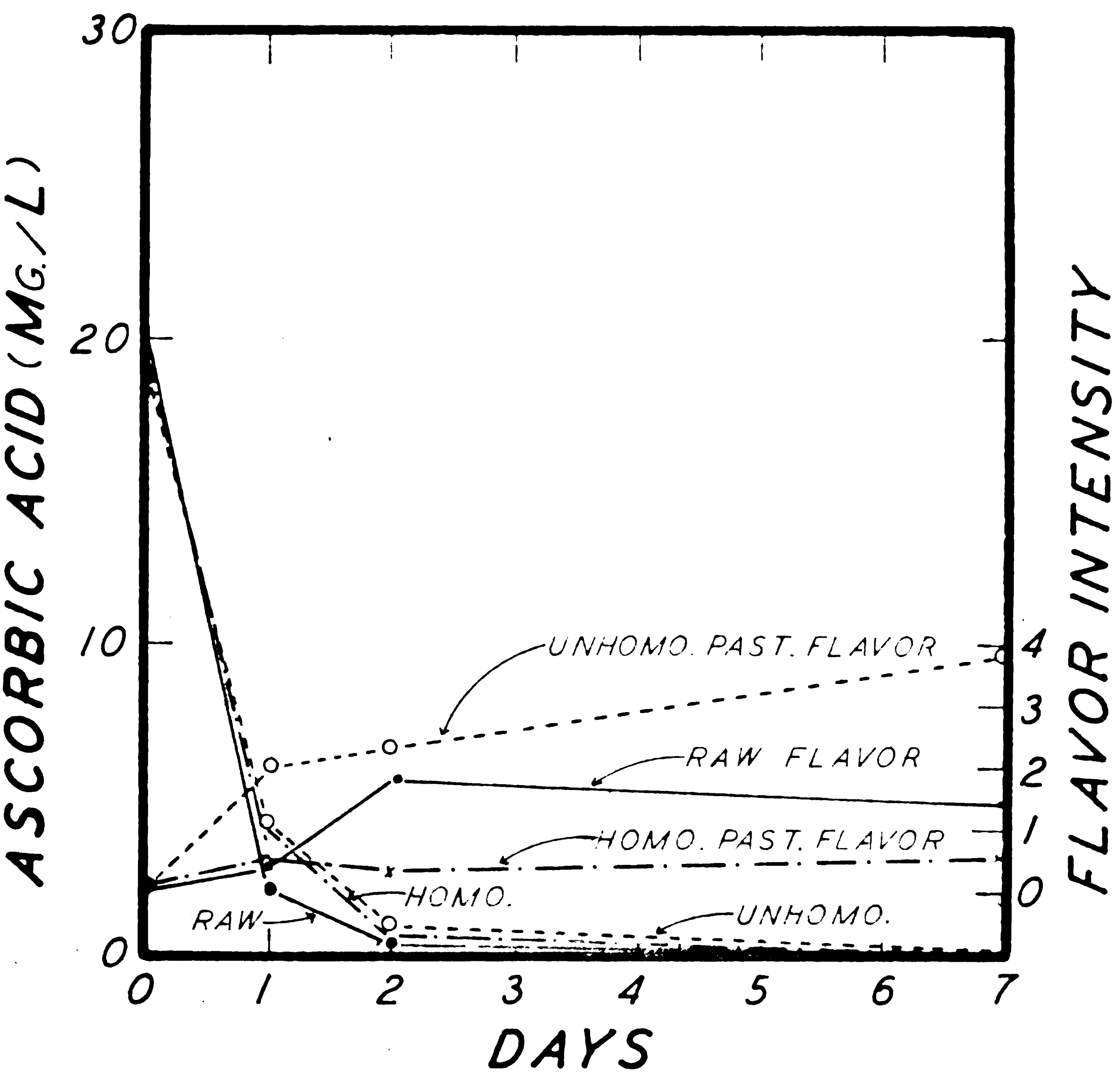

Figure 6. The ascorbic acid content and the development of oxidized flavor in copper-treated raw, unhomogenized pasteurized and homogenized pasteurized milk after various storage periods.

was paralleled by the development of oxidized flavor in the copper-treated unhomogenized pasteurized milk, whereas, in the copper-treated homogenized pasteurized milk very little oxidized flavor developed even though the ascorbic acid disappeared.

Apparently ascorbic acid is not a factor concerned in the resistance of homogenized milk against oxidized flavor development.

The data presented in tables 15 and 16 are shown graphically in figures 5 and 6.

The effect of sunlight upon the oxidation-reduction potential and the development of off flavor in unhomogenized and homogenized milk after various storage periods.

In this experiment oxidation-reduction potentials measurements and flavor studies were made on unhomogenized and homogenized milk in an effort to determine the relationship between oxidation potentials and the development of activated flavor. The oxidation-reduction potential determinations and flavor studies were made on the milk immediately after processing and after exposure to sunlight for 0, 15, 30, 45 and 60 minutes, and after storage in the refrigerator for 1, 6, 24, 48 and 96 hours. The data obtained are presented in tables 17 and 18.

An examination of the data shows that the oxidation-reduction potentials of the samples after exposure to the sun usually decreased on storage. However, in some cases the oxidation-reduction potential increased slightly depending upon the individual sample of milk.

The unhomogenized milk not exposed to the sun showed the smallest decrease in potential on storage. The unhomogenized samples exposed to the sun for 15 minutes showed practically the same trends as did the unexposed sample. The unhomogenized samples exposed to the sun for 30, 40, and 60 minutes showed considerable decreases in oxidation-reduction potentials. The decrease in potential was usually noted at the six-hour observation after being exposed to the sun. A further decrease occurred when the milk was stored for 24, 48 and 96 hours.

The average oxidation-reduction potentials immediately after processing of the unhomogenized samples exposed to the sun for 0, 15, 30, 45, and 60 minutes were 0.3181, 0.3218, 0.3188, 0.3190, and 0.3153 respectively, decreasing to 0.2513, 0.2413, 0.1398, 0.1217 and 0.1383 respectively after 96 hours storage.

The oxidation-reduction potential of the homogenized samples exposed to the sun decreased on storage but the decrease was not so great as in the unhomogenized milk except in the sample exposed to the sun for 15 minutes when the homogenized sample showed a lower potential than did the unhomogenized sample exposed to the sun for the same length of time. An oxidation-reduction potential as low as -0.20 volts was noted in the unhomogenized milk exposed to the sun, whereas the lowest potential noted in the homogenized milk exposed to the sun was -0.05 volts.

An examination of the development of off-flavor in the homogenized milk shows that an activated flavor developed in some milks after only 15 minutes exposure to the sun. Exposure for longer period of time increased the intensity of the activated flavor which could be detected immediately after the exposure period. The intensity of the activated flavor increased on storage in all the homogenized samples which had been exposed to the sun. No activated flavor occurred in any of the homogenized samples not exposed to the sun except in the trial 3 which had been exposed to diffused light for several minutes before storing.

The off-flavor which developed in the unhomogenized milk exposed to the sun was not a definitely oxidized or a true activated flavor but seemed to be a blend of the two flavors. The off-flavor did not develop as quickly or become so intense in the unhomogenized milk exposed to the sun as in the homogenized milk exposed to the sun for a similar period. The off-flavor that developed in the unhomogenized milk exposed to the sun for 45 and 60 minutes was not much greater in intensity then the milk exposed to the sun for only 15 or 30 minutes.

The summarized results of the oxidation-reduction potentials presented in tables 17 and 18 are shown graphically in figures 7 and 8.

Table 17. The effect of sunlight upon the oxidation-reduction potential and the development of off-flavor in unhomogenized milk after various storage periods.

Storage	The oxidation-reduction potential and off-flavor development when homogenized milk was exposed to the sun for									
	0 Minutes		15 Minutes		30 Minutes		45 Minutes		60 Minutes	
	Eh	Flavor	Eh	Flavor	Eh	Flavor	Eh	Flavor	Eh	Flavor
Trial I	July 1, 1939									
*00	0.3076	-	0.3271	-	0.3236	-	0.3241	-	0.3261	-
** 0	0.3090	-	0.3239	-	0.3192	-	0.3185	-	0.3159	-
1	0.3042	-	0.3244	-	0.3151	-	0.3254	-	0.3259	-
6	0.2936	-	0.3281	-	0.3211	-	0.3096	?	0.3381	-
24	0.2896	-	0.3421	-	0.3256	-	0.1606	-	0.3506	-
48	0.2951	-	0.3511	-	0.3191	-	0.0996	-	0.3496	?
96	0.2838	+	0.3303	-	0.1398	-	0.0053	-	0.3613	-
Trial II	July 7, 1939									
00	0.3060	-	0.3080	-	0.3110	-	0.3035	-	0.2995	-
0	0.2976	-	0.3118	-	0.2912	-	0.2857	?	0.3007	?
1	0.2668	-	0.2822	-	0.1894	-	0.2750	?	0.2567	+
6	0.1857	-	0.0092	-	-0.1283	?	0.2097	-	0.1927	?
24	0.0275	?	-0.0820	-	-0.2153	-	-0.1702	++	0.0017	+
48	0.0151	+	-0.1264	-	-0.3406	+	-0.1519	-	-0.0224	-
96	0.0459	++	-0.1230	-	-0.0122	+	-0.1889	-	-0.2251	++
Trial III	July 12, 1939									
00	0.3351	-	0.3251	-	0.3276	-	0.3286	-	0.3271	-
0	0.3409	-	0.3467	-	0.3422	-	0.3292	?	0.2637	+
1	0.3464	-	0.3712	?	0.3516	?	0.3610	+	0.2394	+
6	0.3442	-	0.3858	?	0.3670	?	0.3638	+	0.2656	+
24	0.3520	+++	0.3902	+	0.3644	+	0.3224	++	0.2577	++
48	0.3697	+++	0.4004	+	0.3683	+	0.2967	++	0.2641	+++
96	0.3907	+++	0.3987	++	0.3477	++	0.1614	++	0.1920	++

*00 Determinations made before exposure to the sun.

**0 Determinations made immediately after exposure to the sun.

Table 17. (Continued)

Trial IV	July 19, 1939									
00	0.3236	-	0.3271	-	0.3131	-	0.3199	-	0.3084	-
0	0.3200	-	0.3254	-	0.2927	-	0.3037	-	0.2647	-
1	0.3154	-	0.3278	-	0.2996	-	0.3080	-	0.2442	-
6	0.3154	-	0.3299	++	0.2476	?	0.3117	?	0.2240	++
24	0.3113	++	0.3250	+	0.1631	?	0.3041	+	0.2232	++
48	0.3035	?	0.3070	++	-0.1321	-	0.2706	-	0.1052	?
96	0.2119	-	0.2272	+	-0.1562	+	0.2500	-	-0.0003	-
Trial V	July 28, 1939									
00										
0	0.3007	-	0.3092	-	0.3212	-	0.3332	-	0.3292	-
1	0.2827	-	0.2957	-	0.3117	-	0.3337	-	0.3257	-
6	0.2952	-	0.3092	-	0.3272	?	0.3412	?	0.3437	?
24	0.3068	-	0.3352	-	0.3397	-	0.3437	?	0.3542	?
48	0.3212	-	0.3472	?	0.3680	+	0.3844	+	0.3808	+
96	0.3242	-	0.3732	-	0.3797	-	0.3807	?	0.3637	+
Average of all trials										
00	0.3181	0.00	0.3218	0.00	0.3188	0.00	0.3190	0.00	0.3153	0.00
0	0.3136	0.00	0.3243	0.00	0.3133	0.00	0.3141	0.40	0.2948	0.60
1	0.3031	0.00	0.3203	0.20	0.2935	0.20	0.3206	0.60	0.2784	0.80
6	0.2868	0.00	0.2724	0.30	0.2269	0.30	0.3072	1.00	0.2728	1.40
24	0.2574	1.60	0.2621	0.80	0.1955	0.60	0.1921	1.80	0.2375	1.80
48	0.2599	1.40	0.2559	1.00	0.1165	1.20	0.1799	1.00	0.2155	1.60
96	0.2513	1.80	0.2413	1.00	0.1398	1.40	0.1217	0.80	0.1383	2.00

Table 18. The effect of sunlight upon the oxidation-reduction potential and the development of off-flavor in homogenized milk after various storage periods.

Storage (hours)	The oxidation-reduction potentials and off-flavor development when milk was exposed to the sun for									
	0 Minutes		15 Minutes		30 Minutes		45 Minutes		60 Minutes	
	Eh	Flavor	Eh	Flavor	Eh	Flavor	Eh	Flavor	Eh	Flavor
Trial I	July 1, 1939									
*00	0.3341	-	0.3401	-	0.3556	-	0.3586	-	0.3676	-
**0	0.3355	-	0.3369	?	0.3512	?	0.3532	-	0.3572	+
1	0.3307	-	0.3414	?	0.3596	-	0.3824	-	0.3784	?
6	0.3256	-	0.3376	++	0.3616	-	0.4046	?	0.4036	-
24	0.3231	-	0.3086	++	0.2846	-	0.3966	-	0.3626	+
48	0.3151	-	0.2871	+	0.2226	?	0.3931	?	0.3311	++
96	0.3388	-	0.1243	+	-0.0367	-	0.2678	+	0.1436	+
Trial II	July 7, 1939									
00	0.3305	-	0.3255	-	0.3253	-	0.3209	-	0.3245	-
0	0.3186	-	0.3453	-	0.3462	?	0.3422	+	0.2077	+
1	0.3113	-	0.3332	+	0.3494	?	0.3640	+	0.1452	++
6	0.2972	-	0.2262	+++	0.1764	?	0.3137	+	0.0122	++
24	0.1962	-	0.0745	+	0.0762	+++	0.1935	+++	-0.0307	++
48	0.2335	-	-0.0292	+	0.0363	++	0.1281	++	-0.0027	++
96	0.3377	-	-0.0569	+	0.1010	+	-0.0124	++	-0.0426	++
Trial III	July 12, 1939									
00	0.3226	-	0.3076	-	0.3186	-	0.3256	-	0.3391	-
0	0.3349	?	0.3472	+	0.3481	+	0.2667	++	0.3047	+++
1	0.3279	++	0.3122	++	0.3691	++	0.2755	+++	0.3499	+++
6	0.3325	+++	0.3575	+++	0.3527	+++	0.2663	+++	0.3451	+++
24	0.3556	+++	0.3672	+++	0.3191	+++	0.2032	++	0.3525	+++
48	0.3851	+++	0.3629	+++	0.3076	+++	0.1761	++	0.3604	+++
96	0.3095	+++	0.2817	+++	0.2574	+++	0.1302	++	0.2576	++

* Determinations made before exposure to the sun.

**Determinations made immediately after exposure to the sun.

Table 18. (Continued)

Trial IV July 19, 1939										
00	0.3269	-	0.3312	-	0.3332	-	0.3227	-	0.3332	-
0	0.3225	-	0.3489	-	0.3137	-	0.3417	-	0.3112	?
1	0.3184	-	0.3518	?	0.2941	?	0.3545	?	0.3327	?
6	0.2202	-	0.3514	++	0.2075	++	0.3756	++	0.3607	++
24	0.2127	-	0.3596	+++	0.1788	++	0.3649	+	0.3570	++
48	0.2129	-	0.3292	+++	0.0835	++	0.3426	+	0.3097	++
96	0.1772	-	0.2184	++	0.2184	+++	0.2537	++	0.1327	++
Trial V July 28, 1939										
00										
0	0.2987	-	0.3132	?	0.3262	-	0.3442	?	0.3432	+
1	0.2817	-	0.3177	?	0.3427	?	0.3497	?	0.3457	+
6	0.2832	-	0.3062	++	0.3267	++	0.3422	+	0.3502	++
24	0.2952	-	0.3342	++	0.3512	+++	0.3542	+	0.3662	++
48	0.3302	-	0.3777	+++	0.3917	++	0.3887	+++	0.3887	+++
96	0.3832	-	0.3797	+	0.3827	++	0.3837	++	0.3747	+++
Average of all trials										
00	0.3285	0.00	0.3261	0.00	0.3332	0.00	0.3318	0.00	0.3411	0.00
0	0.3220	0.20	0.3383	0.80	0.3371	1.20	0.3296	1.20	0.3048	2.20
1	0.3140	0.60	0.3313	1.60	0.3430	1.20	0.3452	1.60	0.3104	2.20
6	0.2917	0.80	0.3158	3.40	0.2850	2.20	0.3405	2.40	0.2994	2.60
24	0.2766	0.80	0.2888	3.20	0.2420	3.00	0.3025	2.20	0.2815	3.00
48	0.2554	0.80	0.2655	3.20	0.2083	2.80	0.2657	2.60	0.2774	3.40
96	0.2493	0.80	0.1894	2.60	0.1846	2.40	0.2046	2.80	0.1732	3.00

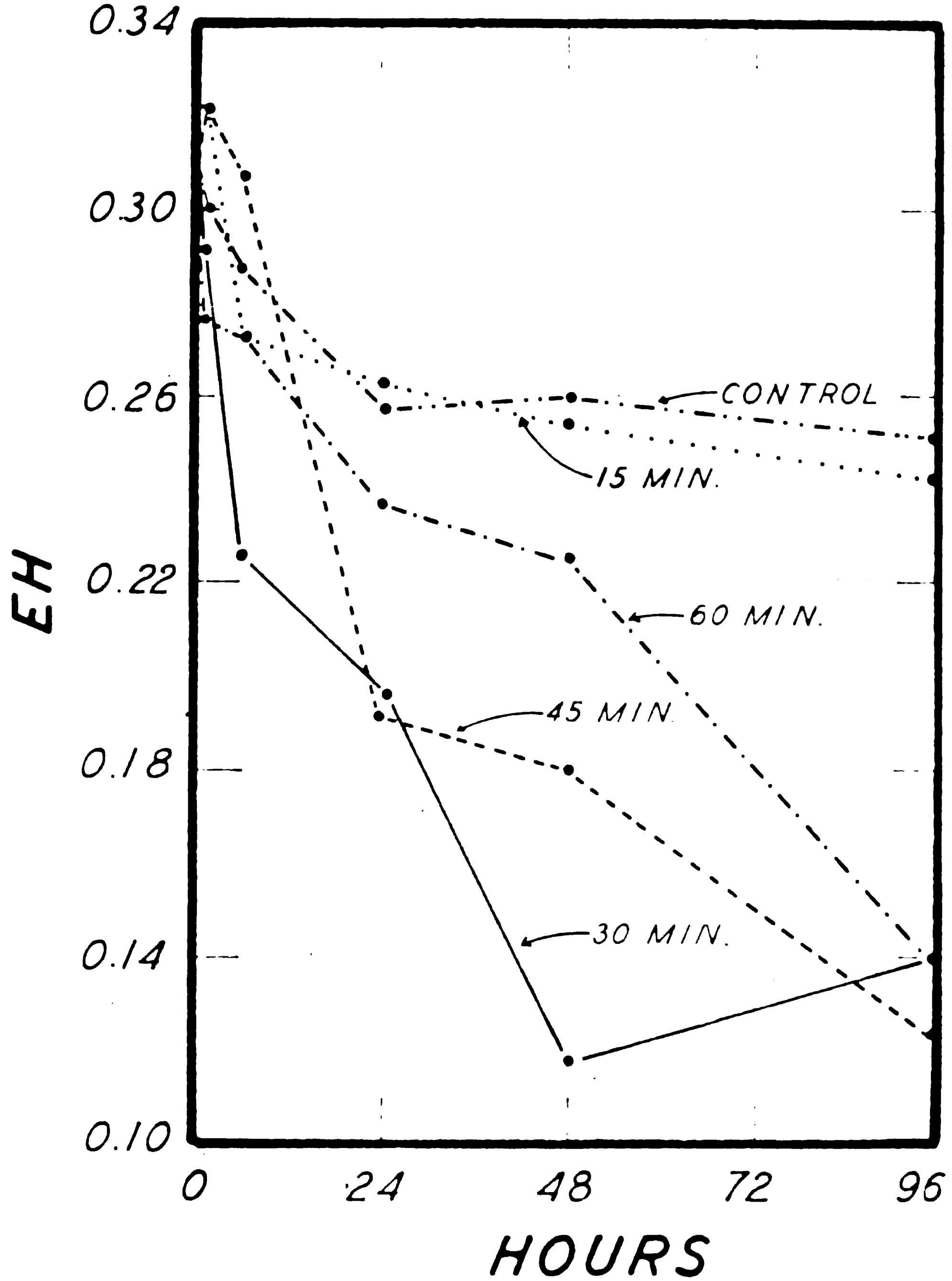

Figure 7. The effect of exposure to sunlight upon the oxidation-reduction potential of unhomogenized milk after various storage periods.

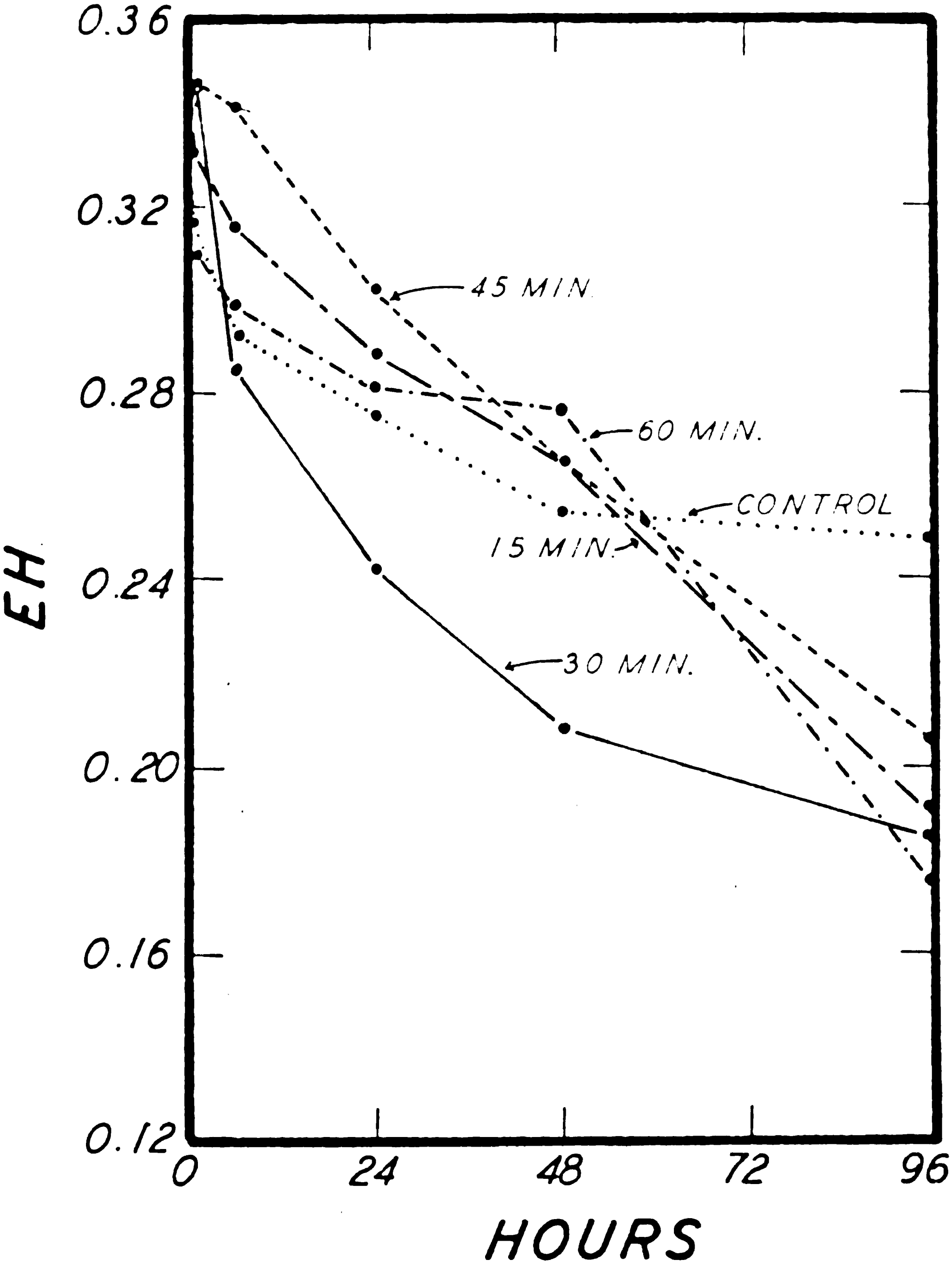

Figure 8. The effect of exposure to sunlight upon the oxidation-reduction potential of homogenized milk after various storage periods.

<u>The development of rancid flavors and increases in titratable acidity due to lipolysis in milks made by mixing raw milk with homogenized pasteurized, homogenized raw milk with homogenized pasteurized and raw milk with homogenized raw milk after various storage periods</u>

In the study of the development of rancidity in homogenized milk the following mixtures of milk were made: 1, unhomogenized raw milk was mixed with homogenized pasteurized at a rate so that samples containing 0, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, 99, and 100 per cent of raw milk were obtained; 2, homogenized raw milk was mixed with homogenized pasteurized milk in the same proportions as stated above; and 3, unhomogenized raw milk was mixed with homogenized raw milk in the same ratios as was the other two series. These samples were titrated for increases in acidity, calculated as lactic acid, and were studied organoleptically for the development of rancid flavor immediately after processing and preparing the various mixtures and after 1, 3, 7 and 10 days of storage at 35° to 40° F. The increase in titratable acidity was determined by subtracting the acidity of the raw milk after the various storage periods from the titratable acidity of the mixtures after similar storage.

<u>Unhomogenized raw milk in homogenized pasteurized milk</u> The data obtained regarding the milks made by mixing unhomogenized raw milk with homogenized pasteurized milk are presented in tables 19, 20 and 21 with the average results presented in table 22 and plotted on figure 9.

An examination of the data shows there was a slight increase in acidity in the homogenized milk after three to five days of storage when it contained as little as one per cent of added unhomogenized raw milk. An increase in acidity of the homogenized pasteurized milk containing 5 per cent of added unhomogenized raw milk could be detected in some samples after one day of storage and as the percentage of unhomogenized raw milk in the homogenized pasteurized milk was increased a corresponding increase in the titratable acidity occurred until a milk containing 50 per cent unhomogenized raw milk and 50 per cent homogenized pasteurized milk was obtained. The maximum increase in acidity occurred when the ratio of unhomogenized raw milk to homogenized pasteurized milk was approximately one to one. As the percentage of unhomogenized raw milk was increased above 50 there was a constant decrease in acidity from the maximum. Small quantities, five and one per cent, of homogenized pasteurized milk in unhomogenized raw milk, sufficient to produce and increase in acidity after one to three days of storage, seemed more effective in producing this increase than similar quantities of unhomogenized raw milk in the homogenized pasteurized milk. The largest increase in acidity usually occurred in the samples containing 50 per cent homogenized pasteurized milk at each of the various storage periods. The increase in acidity due to lipase action upon the fat are apparently equally dependent upon the increased surface caused by homogenization and upon the amount of lipase added by the raw milk.

A rancid flavor could be detected in some samples of homogenized pasteurized milk containing one per cent of unhomogenized raw milk after 7 to 10 days of storage. When the samples contained five per cent of unhomogenized raw milk the rancid flavor developed more readily. A further increase in the percentage of unhomogenized raw milk added to the homogenized pasteurized milk caused a more intense rancid flavor to develop. All the samples containing from 10 to 90 per cent of unhomogenized raw milk developed a very strong rancid flavor especially on storage. When the sample contained less than 10 per cent of homogenized pasteurized milk in unhomogenized raw milk the intensity of the rancid flavor decreased.

Homogenized raw milk in homogenized pasteurized milk The data obtained by mixing homogenized raw milk with homogenized pasteurized milk are presented in tables 23, 24 and 25, with the average results presented in table 26 and shown graphically in figure 10.

An examination of the data shows that the presence of one per cent of homogenized raw milk caused a slight increase in the titratable acidity of some samples after 7 to 10 days of storage. When five per cent of homogenized raw milk was present definite increases in acidity could be detected after the first and third day of storage. As the percentage of homogenized raw milk was increased the titratable acidity increased, with the samples containing 100 per cent of homogenized raw milk showing the largest increases. The acidity increased slightly more rapidly than in the samples studied previously where the raw milk was not homogenized. This is reasonable because all the fat in the latter mixtures had been subjected to homogenization and therefore most of the fat globules would be decreased in size, thus, there would be more surface upon which the lipase could act, whereas, in the samples composed of unhomogenized raw milk and homogenized pasteurized milk only a part of each sample had been subjected to homogenization. Consequently, all the fat globules had not been decreased in size so there was not as much surface exposed to the activity of the lipase. Another important difference between the milk composed of unhomogenized raw and homogenized pasteurized milk and the milk composed of homogenized raw milk and homogenized pasteurized milk is the fact that in the former the maximum acidity occurred when a 1 to 1 mixture was present after which the increase of titratable acidity decreased; whereas, in the latter the acidity reached a maximum when the sample consisted of

100 per cent of homogenized raw milk. The development of a rancid flavor closely followed the changes in titratable acidity.

Unhomogenized raw milk in homogenized raw milk. The data obtained regarding the development of rancidity in mixtures of unhomogenized raw milk and homogenized raw milk are presented in tables 27 and 28, with the average results presented in table 29.

An examination of the data show the same general trends in the development of a rancid flavor and increases in acidity as in the milk composed of homogenized raw milk and homogenized pasteurized milk, except that the acidity increased slightly more rapidly as the percentage of homogenized raw milk in unhomogenized raw milk was increased up to approximately 50 oer cent, after which the increase in titratable acidity was not so rapid.

The average results in table 29 are presented graphically in figure 11.

Table 19. The development of rancid flavors and increases in titratable acidity due to lipolysis in milk made by mixing unhomogenized raw milk with homogenized pasteurized milk after various storage periods. (Trial 1)

Sample: Unhomo. Raw (%)	Sample: Homo. Pasteurized (%)	1 day: Acidity (%)	1 day: Flavor	3 days: Acidity (%)	3 days: Flavor	7 days: Acidity (%)	7 days: Flavor	10 days: Acidity (%)	10 days: Flavor
0	100	0.0000	-	0.0000	-	0.0000	-	0.0000	-
1	99	0.0000	-	0.0041	-	0.0000	?	0.0000	+
5	95	0.0041	-	0.0102	?	0.0111	+	0.0245	++
10	90	0.0061	?	0.0225	?	0.0222	++	0.0378	+++
20	80	0.0144	+	0.0348	++	0.0378	+++	0.0512	+++
30	70	0.0246	+	0.0451	+++	0.0534	+++	0.0623	+++
40	60	0.0287	++	0.0531	+++	0.0623	+++	0.0756	+++
50	50	0.0328	++	0.0492	+++	0.0667	+++	0.0801	+++
60	40	0.0347	++	0.0574	+++	0.0623	+++	0.0778	+++
70	30	0.0347	++	0.0574	+++	0.0667	+++	0.0778	+++
80	20	0.0307	++	0.0471	++	0.0534	+++	0.0589	+++
90	10	0.0144	+	0.0225	+	0.0222	+++	0.0311	+++
95	5	0.0041	?	0.0102	?	0.0067	+	0.0156	+
99	1	0.0041	-	0.0041	-	0.0000	?	0.0000	?
100	0	0.0000	-	0.0000	-	0.0000	-	0.0000	-

Table 20. <u>The development of rancid flavors and increases in titratable acidity due to lipolysis in milk made by mixing unhomogenized raw milk with homogenized pasteurized milk after various storage periods. (Trial 2)</u>

Sample		Increases in acidity and rancidity after							
Unhomo. Raw (%)	Homo. Pasteurized (%)	1 day Acidity (%)	1 day Flavor	3 days Acidity (%)	3 days Flavor	7 days Acidity (%)	7 days Flavor	10 days Acidity (%)	10 days Flavor
0	100	0.0045	-	0.0067	-	0.0045	-	0.0000	-
1	99	0.0045	-	0.0089	-	0.0067	-	0.0042	-
5	95	0.0133	-	0.0178	-	0.0245	?	0.0220	++
10	90	0.0178	-	0.0245	?	0.0378	+	0.0420	++
20	80	0.0245	+	0.0334	+	0.0489	++	0.0575	+++
30	70	0.0289	+	0.0467	++	0.0600	+++	0.0664	+++
40	60	0.0311	++	0.0467	+++	0.0589	+++	0.0731	+++
50	50	0.0356	++	0.0512	+++	0.0667	+++	0.0820	+++
60	40	0.0356	++	0.0512	+++	0.0589	+++	0.0709	+++
70	30	0.0334	++	0.0489	+++	0.0589	+++	0.0664	+++
80	20	0.0311	++	0.0424	++	0.0400	++	0.0420	++
90	10	0.0178	+	0.0245	+	0.0267	+	0.0264	++
95	5	0.0111	?	0.0133	-	0.0178	-	0.0131	+
99	1	0.0111	-	0.0089	-	0.0067	-	0.0086	-
100	0	0.0000	-	0.0000	-	0.0000	-	0.0000	-

Table 21. The development of rancid flavors and increases in titratable acidity due to lipolysis in milk made by mixing unhomogenized raw milk with homogenized pasteurized milk after various storage periods. (Trial 3)

Sample		Increases in acidity and rancidity after							
Unhomo. Raw (%)	Homo. Pasteurized (%)	1 day Acidity (%)	1 day Flavor	3 days Acidity (%)	3 days Flavor	7 days Acidity (%)	7 days Flavor	10 days Acidity (%)	10 days Flavor
0	100	0.0000	-	0.0000	-	0.0000	-	0.0000	-
1	99	0.0000	-	0.0000	-	0.0022	-	0.0000	-
5	95	0.0000	-	0.0045	-	0.0178	-	0.0311	+
10	90	0.0022	-	0.0200	?	0.0289	+	0.0534	++
20	80	0.0156	+	0.0289	++	0.0489	++	0.0667	++
30	70	0.0245	+	0.0445	++	0.0667	+++	0.0801	+++
40	60	0.0311	?	0.0512	+++	0.0712	+++	0.0734	+++
50	50	0.0400	+	0.0534	+++	0.0801	+++	0.0823	+++
60	40	0.0378	+	0.0556	+++	0.0778	+++	0.0712	+++
70	30	0.0311	+	0.0534	+++	0.0689	+++	0.0712	+++
80	20	0.0289	+	0.0467	++	0.0556	++	0.0512	+++
90	10	0.0222	+	0.0356	+	0.0423	++	0.0378	++
95	5	0.0134	-	0.0222	-	0.0356	+	0.0289	+
99	1	0.0022	-	0.0089	-	0.0068	-	0.0000	-
100	0	0.0000	-	0.0000	-	0.0000	-	0.0000	-

Table 22. The development of rancid flavors and increases in titratable acidity due to lipolysis in milk made by mixing unhomogenized raw milk with homogenized pasteurized milk after various storage periods. (Average of all trials)

Sample		Increases in acidity and rancidity after							
Unhomo.	Homo.	1 day		3 days		7 days		10 days	
Raw (%)	Pasteurized (%)	Acidity (%)	Flavor	Acidity (%)	Flavor	Acidity (%)	Flavor	Acidity (%)	Flavor
0	100	0.0015	0.00	0.0022	0.00	0.0015	0.00	0.0000	0.00
1	99	0.0015	0.00	0.0044	0.00	0.0030	0.33	0.0014	0.67
5	95	0.0058	0.00	0.0109	0.33	0.0178	1.00	0.0225	2.67
10	90	0.0087	0.33	0.0223	1.00	0.0297	2.33	0.0444	2.67
20	80	0.0181	2.00	0.0323	2.67	0.0452	3.33	0.0585	3.67
30	70	0.0260	2.00	0.0454	3.00	0.0500	3.33	0.0699	3.67
40	60	0.0303	2.33	0.0503	4.00	0.0675	4.00	0.0740	4.00
50	50	0.0361	2.67	0.0512	4.00	0.0712	4.00	0.0814	4.00
60	40	0.0360	2.67	0.0547	4.00	0.0697	4.00	0.0733	4.00
70	30	0.0331	2.33	0.0532	4.00	0.0682	4.00	0.0711	4.00
80	20	0.0303	2.67	0.0454	3.00	0.0497	3.33	0.0540	3.67
90	10	0.0181	2.00	0.0275	2.00	0.0304	3.00	0.0312	3.33
95	5	0.0095	0.67	0.0153	0.33	0.0200	1.33	0.0192	3.00
99	1	0.0058	0.00	0.0073	0.00	0.0045	0.33	0.0029	0.33
100	0	0.0000	0.00	0.0000	0.00	0.0000	0.00	0.0000	0.00

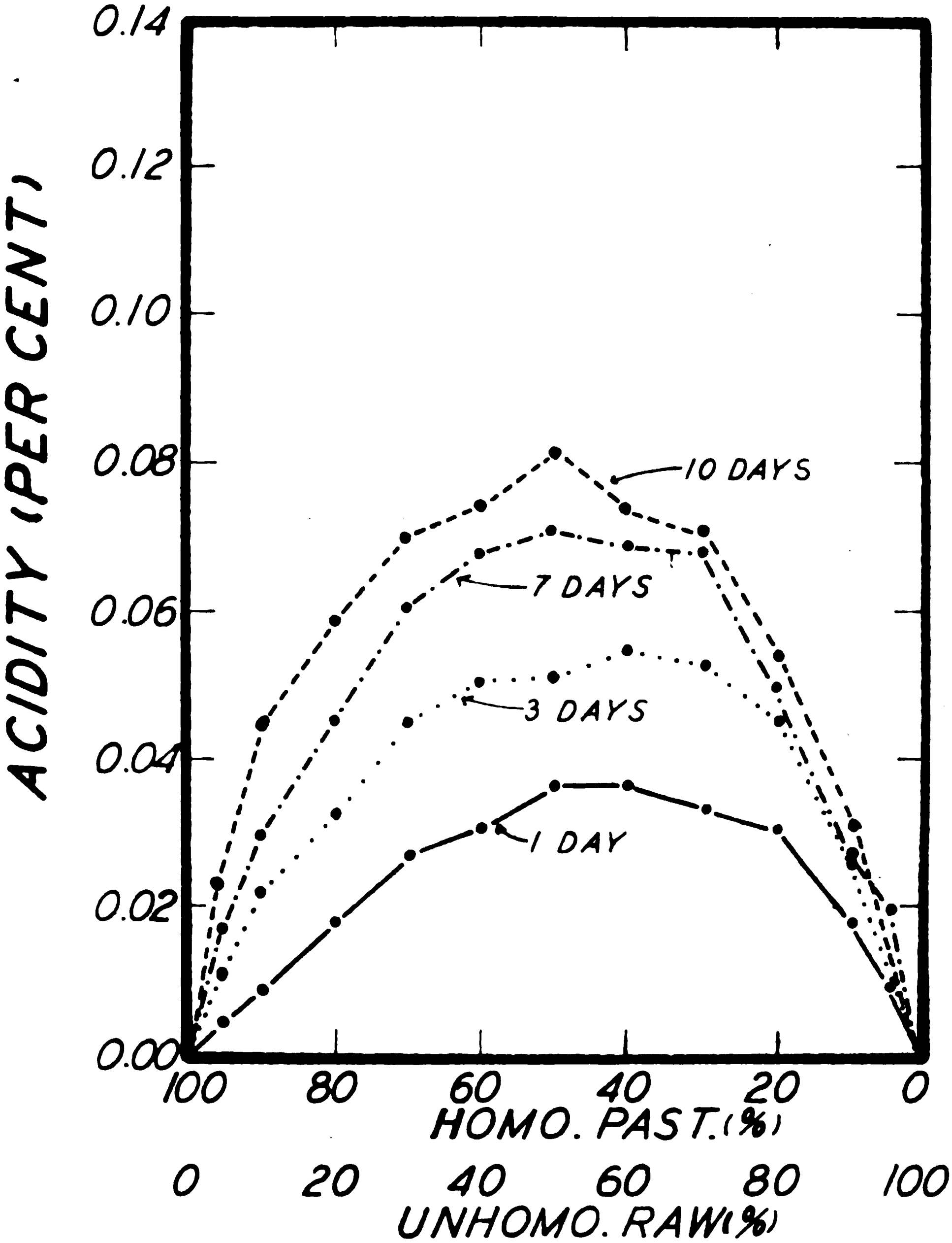

Figure 9. The increases in acidity after different storage periods when unhomogenized raw milk was added to homogenized pasteurized milk in different proportions.

Table 23. The development of rancid flavors and the increase in titratable acidity due to lipolysis in milk made by mixing homogenized raw milk with homogenized pasteurized milk after various storage periods. (Trial 1)

Sample		Increases in acidity and rancidity after									
Homo. Raw (%)	Homo. Pasteurized (%)	0 day Acidity (%)	0 day Flavor	1 day Acidity (%)	1 day Flavor	3 days Acidity (%)	3 days Flavor	7 days Acidity (%)	7 days Flavor	10 days Acidity (%)	10 days Flavor
0	100	0.0020	-	0.0000	-	0.0000	-	0.0000	-	0.0000	-
1	99	0.0000	-	0.0000	-	0.0000	-	0.0000	-	0.0022	+
5	95	0.0082	-	0.0062	-	0.0062	+	0.0178	+	0.0200	+
10	90	0.0062	-	0.0102	-	0.0246	+	0.0289	++	0.0378	+++
20	80	0.0082	-	0.0225	?	0.0369	+	0.0400	+++	0.0534	+++
30	70	0.0103	-	0.0287	?	0.0451	++	0.0534	+++	0.0623	+++
40	60	0.0144	-	0.0348	++	0.0531	++	0.0756	+++	0.0823	+++
50	50	0.0185	-	0.0451	++	0.0615	++	0.0823	+++	0.0912	+++
60	40	0.0205	-	0.0451	+++	0.0676	++	0.0867	+++	0.1001	+++
70	30	0.0185	-	0.0574	+++	0.0800	++	0.1023	+++	0.1112	+++
80	20	0.0205	-	0.0531	+++	0.0840	+++	0.1023	+++	0.1179	+++
90	10	0.0246	-	0.0615	+++	0.0902	+++	0.1090	+++	0.1290	+++
95	5	0.0205	-	0.0635	+++	0.0963	+++	0.1156	+++	0.1290	+++
99	1	0.0226	-	0.0717	+++	0.1004	+++	0.1201	+++	0.1334	+++
100	0	0.0205	-	0.0758	+++	0.1004	+++	0.1156	+++	0.1334	+++

Table 24. The development of rancid flavors and the increase in titratable acidity due to lipolysis in milk made by mixing homogenized raw milk with homogenized pasteurized milk after various storage periods. (Trial 2)

Sample		Increases in acidity and rancidity after									
		0 days		1 day		3 days		7 days		10 days	
Homo. Raw (%)	Homo. Pasteurized (%)	Acidity (%)	Flavor	Acidity (%)	Flavor	Acidity (%)	Flavor	Acidity (%)	Flavor	Acidity (%)	Flavor
0	100	0.0000	-	0.0045	-	0.0067	-	0.0045	-	0.0000	-
1	99	0.0000	-	0.0045	-	0.0000	-	0.0089	-	0.0042	-
5	95	0.0041	-	0.0089	-	0.0178	?	0.0245	?	0.0197	++
10	90	0.0021	-	0.0133	-	0.0245	?	0.0334	++	0.0353	++
20	80	0.0082	-	0.0267	-	0.0356	++	0.0512	++	0.0531	+++
30	70	0.0102	-	0.0267	?	0.0512	++	0.0623	+++	0.0709	+++
40	60	0.0102	-	0.0445	?	0.0512	+++	0.0823	+++	0.0842	+++
50	50	0.0164	-	0.0378	+	0.0623	+++	0.0867	+++	0.0953	+++
60	40	0.0123	-	0.0467	++	0.0623	+++	0.0934	+++	0.0976	+++
70	30	0.0184	-	0.0489	++	0.0712	+++	0.1001	+++	0.1109	+++
80	20	0.0205	-	0.0578	++	0.0778	+++	0.1067	+++	0.1198	+++
90	10	0.0205	-	0.0600	++	0.0823	+++	0.1134	+++	0.1243	+++
95	5	0.0225	-	0.0623	++	0.0845	+++	0.1179	+++	0.1309	+++
99	1	0.0184	-	0.0689	+++	0.0867	+++	0.1179	+++	0.1332	+++
100	0	0.0205	-	0.0689	+++	0.0845	+++	0.1179	+++	0.1354	+++

Table 25. The development of rancid flavors and the increase in titratable acidity due to lipolysis in milk made by mixing homogenized raw milk with homogenized pasteurized milk after various storage periods. (Trial 3)

Sample		Increases in acidity and rancidity after									
		0 days		1 day		3 days		7 days		10 days	
Homo. Raw (%)	Homo. Pasteurized (%)	Acidity (%)	Flavor	Acidity (%)	Flavor	Acidity (%)	Flavor	Acidity (%)	Flavor	Acidity (%)	Flavor
0	100	0.0000	-	0.0000	-	0.0000	-	0.0000	-	0.0000	-
1	99	0.0000	-	0.0000	-	0.0000	-	0.0000	-	0.0067	-
5	95	0.0000	-	0.0000	-	0.0045	?	0.0245	+	0.0311	+
10	90	0.0000	-	0.0067	-	0.0278	+	0.0356	++	0.0512	++
20	80	0.0000	-	0.0156	++	0.0311	++	0.0578	+++	0.0778	+++
30	70	0.0044	-	0.0311	+	0.0445	++	0.0712	+++	0.0867	+++
40	60	0.0111	-	0.0378	+	0.0534	+++	0.0756	+++	0.0934	+++
50	50	0.0067	-	0.0445	++	0.0667	+++	0.0867	+++	0.0978	+++
60	40	0.0089	-	0.0467	++	0.0712	+++	0.0934	+++	0.1112	+++
70	30	0.0178	-	0.0512	++	0.0778	+++	0.1045	+++	0.1134	+++
80	20	0.0156	-	0.0534	+++	0.0823	+++	0.1112	+++	0.1201	+++
90	10	0.0133	-	0.0601	+++	0.0867	+++	0.1134	+++	0.1223	+++
95	5	0.0245	-	0.0623	+++	0.0912	+++	0.1201	+++	0.1379	+++
99	1	0.0178	-	0.0623	+++	0.0978	+++	0.1245	+++	0.1379	+++
100	0	0.0267	-	0.0755	+++	0.0934	+++	0.1312	+++	0.1357	+++

Table 26. The development of rancid flavors and the increases in titratable acidity due to lipolysis in milk made by mixing homogenized raw milk with homogenized pasteurized milk after various storage period. (Average of all trials)

Sample		Increases in acidity and rancidity after									
Homo. Raw (%)	Homo. Pasteurized (%)	0 days Acidity (%)	0 days Flavor	1 day Acidity (%)	1 day Flavor	3 days Acidity (%)	3 days Flavor	7 days Acidity (%)	7 days Flavor	10 days Acidity (%)	10 days Flavor
0	100	0.0007	0.00	0.0014	0.00	0.0022	0.00	0.0014	0.00	0.0000	0.00
1	99	0.0000	0.00	0.0014	0.00	0.0000	0.00	0.0030	0.00	0.0044	0.67
5	95	0.0041	0.00	0.0050	0.00	0.0095	1.33	0.0222	1.66	0.0236	2.33
10	90	0.0027	0.00	0.0101	0.00	0.0256	1.66	0.0326	3.00	0.0414	3.33
20	80	0.0055	0.00	0.0219	1.33	0.0345	2.67	0.0497	3.67	0.0614	4.00
30	70	0.0083	0.00	0.0288	1.33	0.0469	3.00	0.0623	4.00	0.0733	4.00
40	60	0.0119	0.00	0.0390	2.00	0.0525	3.67	0.0778	4.00	0.0866	4.00
50	50	0.0138	0.00	0.0425	2.67	0.0635	3.67	0.0852	4.00	0.0948	4.00
60	40	0.0139	0.00	0.0462	3.33	0.0670	3.67	0.0912	4.00	0.1029	4.00
70	30	0.0182	0.00	0.0525	3.33	0.0753	3.67	0.1023	4.00	0.1118	4.00
80	20	0.0188	0.00	0.0538	3.67	0.0814	4.00	0.1067	4.00	0.1193	4.00
90	10	0.0195	0.00	0.0605	3.67	0.0864	4.00	0.1119	4.00	0.1252	4.00
95	5	0.0225	0.00	0.0627	3.67	0.0907	4.00	0.1178	4.00	0.1326	4.00
99	1	0.0196	0.00	0.0676	4.00	0.0950	4.00	0.1208	4.00	0.1348	4.00
100	0	0.0226	0.00	0.0734	4.00	0.0928	4.00	0.1216	4.00	0.1348	4.00

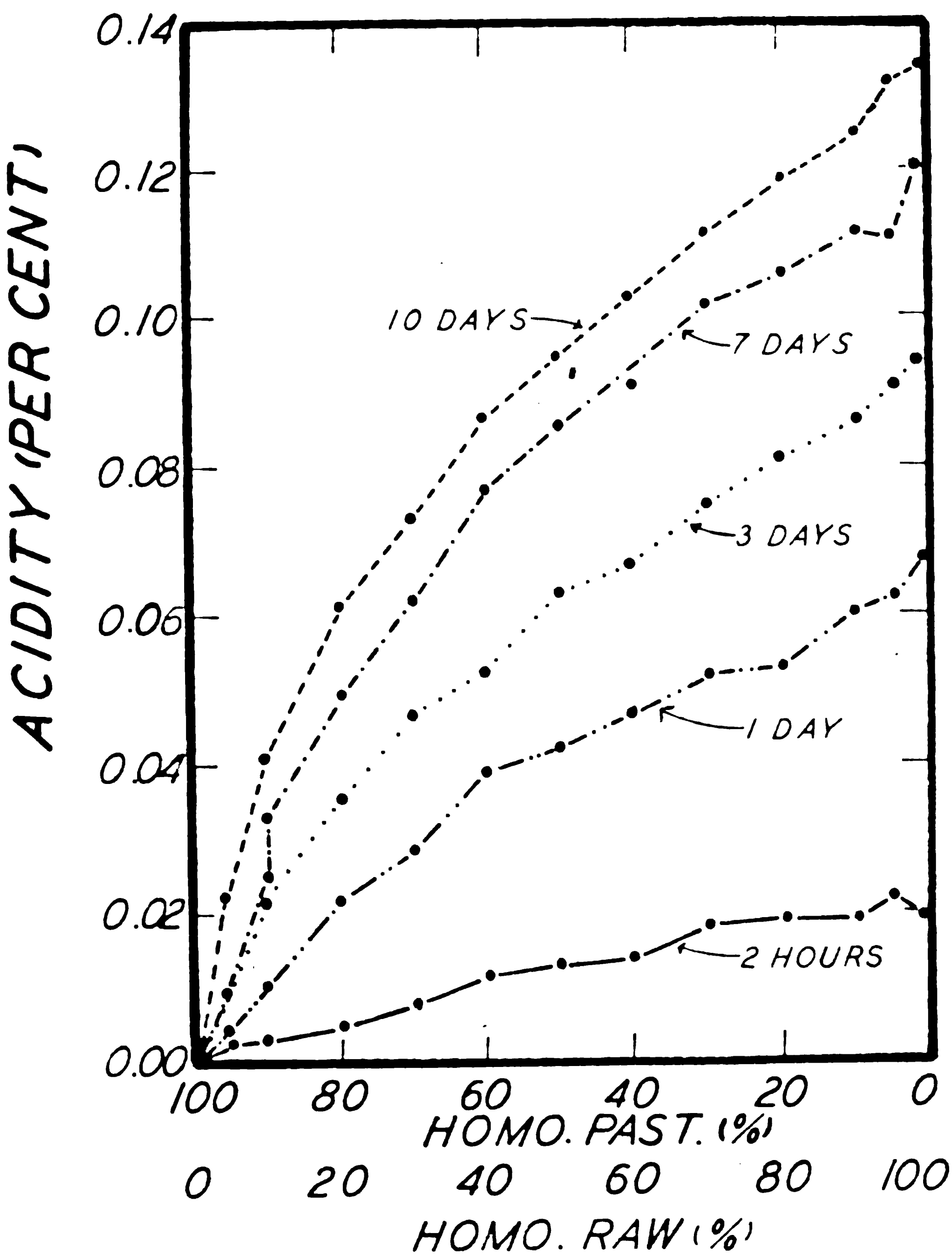

Figure 10. The increases in acidity after different storage periods when homogenized raw milk was added to homogenized pasteurized milk in different proportions.

Table 27. The development of rancid flavors and increases in titratable acidity due to lipolysis in milk made by mixing raw milk with homogenized raw milk after various storage periods. (Trial 1)

Sample		Increases in acidity and rancidity after									
Homo. Raw (%)	Unhomo. Raw (%)	0 days Acidity (%)	0 days Flavor	1 day Acidity (%)	1 day Flavor	3 days Acidity (%)	3 days Flavor	7 days Acidity (%)	7 days Flavor	10 days Acidity (%)	10 days Flavor
0	100	0.0000	-	0.0000	-	0.0000	-	0.0000	-	0.0000	-
1	99	0.0000	-	0.0067	-	0.0067	-	0.0111	-	0.0512	-
5	95	0.0000	-	0.0111	-	0.0267	?	0.0378	?	0.0400	+
10	90	0.0000	-	0.0267	?	0.0336	+	0.0445	+	0.0578	++
20	80	0.0000	-	0.0378	+	0.0534	++	0.0734	++	0.0867	+++
30	70	0.0045	-	0.0445	+	0.0578	+++	0.0889	+++	0.1134	+++
40	60	0.0067	-	0.0489	++	0.0712	+++	0.1045	+++	0.1134	+++
50	50	0.0067	-	0.0578	++	0.0778	+++	0.1067	+++	0.1290	+++
60	40	0.0111	-	0.0512	++	0.0956	+++	0.1156	+++	0.1179	+++
70	30	0.0156	-	0.0578	+++	0.0890	+++	0.1179	+++	0.1312	+++
80	20	0.0178	-	0.0601	+++	0.0845	+++	0.1245	+++	0.1423	+++
90	10	0.0178	-	0.0578	+++	0.0934	+++	0.1268	+++	0.1334	+++
95	5	0.0200	-	0.0645	+++	0.0912	+++	0.1290	+++	0.1357	+++
99	1	0.0200	-	0.0645	+++	0.0962	+++	0.1245	+++	0.1334	+++
100	0	0.0267	-	0.0756	+++	0.0934	+++	0.1312	+++	0.1357	+++

Table 28. The development of rancid flavors and increases in titratable acidity due to lipolysis in milk made by mixing raw milk with homogenized raw milk after various storage periods. (Trial 2)

Sample		Increases in acidity and rancidity after									
Homo. Raw (%)	Unhomo. Raw (%)	0 days Acidity (%)	0 days Flavor	1 day Acidity (%)	1 day Flavor	3 days Acidity (%)	3 days Flavor	7 days Acidity (%)	7 days Flavor	10 days Acidity (%)	10 days Flavor
0	100	0.0000	-	0.0000	-	0.0000	-	0.0000	-	0.0000	-
1	99	0.0045	-	0.0000	-	0.0000	-	0.0000	-	0.0067	-
5	95	0.0067	-	0.0111	-	0.0067	?	0.0000	?	0.0156	+
10	90	0.0089	-	0.0133	+	0.0200	+	0.0133	+	0.0267	+
20	80	0.0089	-	0.0267	+	0.0423	+	0.0356	++	0.0467	++
30	70	0.0111	-	0.0400	+	0.0600	++	0.0600	++	0.0734	++
40	60	0.0111	-	0.0467	++	0.0734	+++	0.0734	+++	0.0845	++
50	50	0.0156	-	0.0534	++	0.0712	+++	0.0867	+++	0.0934	+++
60	40	0.0178	-	0.0534	++	0.0845	+++	0.0845	+++	0.0956	+++
70	30	0.0222	-	0.0556	+++	0.0934	+++	0.0934	+++	0.1023	+++
80	20	0.0178	-	0.0623	+++	0.0934	+++	0.0934	+++	0.1023	+++
90	10	0.0200	-	0.0600	+++	0.0912	+++	0.0978	+++	0.1112	+++
95	5	0.0289	-	0.0600	+++	0.1001	+++	0.1001	+++	0.1112	+++
99	1	0.0267	-	0.0623	+++	0.1023	+++	0.1023	+++	0.1112	+++
100	0	0.0267	-	0.0645	+++	0.1023	+++	0.0912	+++	0.1201	+++

Table 29. The development of rancid flavors and increases in titratable acidity due to lipolysis in milk made by mixing raw milk with homogenized raw milk after various storage periods. (Average of all trials)

Sample		Increases in acidity and rancidity after									
Homo. Raw (%)	Unhomo. Raw (%)	0 days Acidity (%)	0 days Flavor	1 day Acidity (%)	1 day Flavor	3 days Acidity (%)	3 days Flavor	7 days Acidity (%)	7 days Flavor	10 days Acidity (%)	10 days Flavor
0	100	0.0000	0.00	0.0000	0.00	0.0000	0.00	0.0000	0.00	0.0000	0.00
1	99	0.0022	0.00	0.0033	0.00	0.0033	0.00	0.0056	0.00	0.0289	0.00
5	95	0.0033	0.00	0.0111	0.00	0.0167	1.00	0.0189	1.00	0.0278	2.00
10	90	0.0045	0.00	0.0200	1.50	0.0268	2.00	0.0289	2.00	0.0423	2.50
20	80	0.0045	0.00	0.0323	2.00	0.0478	2.50	0.0550	3.00	0.0667	3.50
30	70	0.0078	0.00	0.0423	2.00	0.0589	3.50	0.0750	3.50	0.0934	3.50
40	60	0.0089	0.00	0.0478	2.00	0.0723	4.00	0.0890	4.00	0.0990	4.00
50	50	0.0111	0.00	0.0556	2.00	0.0745	4.00	0.0967	4.00	0.1112	4.00
60	40	0.0145	0.00	0.0523	2.00	0.0901	4.00	0.1001	4.00	0.1067	4.00
70	30	0.0189	0.00	0.0567	4.00	0.0912	4.00	0.1056	4.00	0.1168	4.00
80	20	0.0178	0.00	0.0612	4.00	0.0900	4.00	0.1030	4.00	0.1223	4.00
90	10	0.0190	0.00	0.0589	4.00	0.0923	4.00	0.1123	4.00	0.1223	4.00
95	5	0.0245	0.00	0.0623	4.00	0.0956	4.00	0.1145	4.00	0.1234	4.00
99	1	0.0234	0.00	0.0634	4.00	0.0990	4.00	0.1134	4.00	0.1223	4.00
100	0	0.0267	0.00	0.0701	4.00	0.0978	4.00	0.1112	4.00	0.1279	4.00

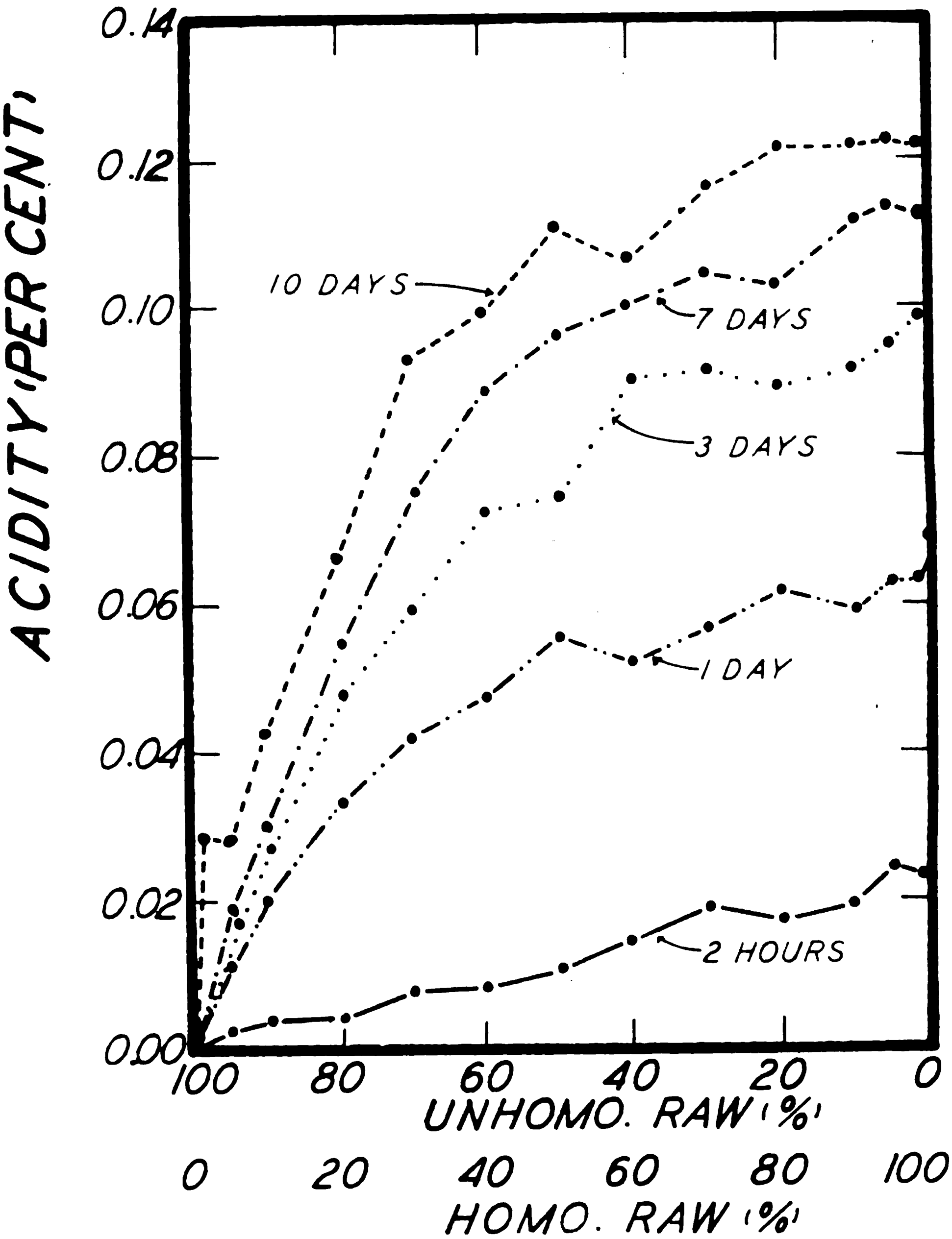

Figure 11. The increases in acidity after different storage periods when unhomogenized raw milk was added to homogenized raw milk in different proportions.

DISCUSSION

Milk from different sources studied in this experiment showed variations in oxidation-reduction potentials immediately after processing and after storage for various periods. The potential of the milk increased on storage and reached a maximum on approximately the fourteenth day of storage after which there was a tendency for the oxidation-reduction potential to decrease. However, Tracy et al (1933) found that freshly drawn milk had a tendency to go toward reduction on storage for 24 hours, but they did not carry their work over long storage periods. There was no significant difference in the oxidation-reduction potential of unhomogenized and homogenized immediately after processing or during the storage periods. This is in agreement with the work of Tracy et al (1933).

A rise in the oxidation reduction-potential during storage of the unhomogenized milk was accompanied by the development of an oxidized flavor. However, an increase in oxidation-reduction potential in the homogenized milk was not accompanied by the development of an oxidized flavor. The stability of homogenized milk against oxidized flavor cannot be explained on an oxidation-reduction basis.

The homogenization of pasteurized milk at a pressure of 2500 pounds was sufficient to prevent the development of oxidized flavor in milk stored for as long as 35 days. An oxidized flavor developed in only a few of the homogenized samples which were studied. These results are in harmony with the work of Tracy et al (1933), Doan and Minster (1933), Ross (1937) and Trout and Gould (1938) who usually employed storage periods of only 48 to 72 hours.

The addition of copper to milk increased the oxidation-reduction potential of both the unhomogenized milk and the homogenized milk. The potential of the copper-treated milk increased rapidly on storage, reaching a maximum potential after one day of storage. After reaching the maximum the potential had a tendency to decrease.

There was a close relationship between an increase in the oxidation-reduction potential and the development of oxidized flavor in the unhomogenized copper-treated milk. The relationship was not very close between the development of oxidized flavor and an increase in the oxidation-reduction potential of copper-treated homogenized milk, again showing that the flavor stability of homogenized milk cannot be explained on an oxidation-reduction basis.

The exposure of unhomogenized milk and homogenized milk to sunlight resulted in a decrease in oxidation-reduction potential. Whitehead (1931) observed similar changes in the oxidation-reduction potential when milk was exposed to the sun. An activated flavor developed more rapidly in the homogenized milk on exposure to sunlight than in the unhomogenized milk similarly exposed. The fact that the oxidation-reduction potential of the unhomogenized milk decreased as a rule when an oxidized flavor developed, whereas, the potential of milk decreased when an activated flavor developed, seems to indicate further that there is a distinct difference in the oxidized flavor and the activated flavor.

The removal of the membrane from the fat globule by churning the cream and washing the fat with water seemed to increase the stability of the remade homogenized milk against oxidized flavor development as compared with the original unhomogenized milk. The remade homogenized milk in which the fat had been churned and washed three times was more resistant to oxidized flavor development than the remade milk in which the fat had been churned and washed only one or two times. The probable reason for the greater stability of the samples washed three times was the removal of most of the lecithin from the milk by the churning and washing. Considerable evidence has been presented in the literature indicating that lecithin is the substance which becomes oxidized and causes the oxidized flavor, and the removal of the lecithin from the milk results in a milk more stable against oxidized flavor. The lecithin content of the buttermilk from each of the churnings decreased with each successive churning showing that some of the lecithin was removed from the milk by churning and washing.

On the other hand, the milk in which the fat had been churned and washed one, two or three times was more susceptible to the development of oxidized flavor than the normal homogenized milk. This greater susceptibility of the washed samples toward oxidized flavor was probably due to the severe treatment such as churning, washing and homogenization to which the fat was subjected.

Milk reconstituted from homogenized cream and homogenized skim milk was more resistant toward oxidized flavor development than milks made from homogenized cream and unhomogenized skim milk, unhomogenized cream and homogenized skim milk, or from unhomogenized cream and unhomogenized skim milk. The milk made from homogenized cream and unhomogenized skim milk was less susceptible to oxidized flavor development than the milk made from unhomogenized cream and homogenized skim milk, indicating that the substance or substances affected by homogenization in such manner as to prevent oxidized flavor development are more closely associated with the cream than with the skim milk. However, the milk made from unhomogenized cream and homogenized skim milk was more resistant to oxidized flavor development than the milk reconstituted from cream and skim milk which were not homogenized or than the original unhomogenized milk. This possibly indicates that some substance or substances in the skim milk are affected also by homogenization in such a manner as to retard oxidized flavor development. The normal homogenized milk was more stable against oxidized flavor development than any of the reconstituted samples showing that maximum stability was obtained only when the cream and skim milk were in their original form as whole milk while being homogenized.

The ascorbic acid content of unhomogenized pasteurized and homogenized pasteurized copper-free milk immediately after processing was lower than that of the fresh raw milk. The ascorbic acid content of the raw and homogenized pasteurized milk decreased more rapidly upon storage than that of the unhomogenized pasteurized milk. The ascorbic acid disappeared more rapidly in the homogenized pasteurized milk than in the raw or unhomogenized pasteurized milk. In copper-treated milk the rate of disappearance of the ascorbic acid in unhomogenized and homogenized milk were similar. Woessner and associates (1939) observed that homogenization tended to destroy the ascorbic acid in commercial bottled milk.

The homogenized samples treated with copper were not very susceptible to oxidized flavor development even though the ascorbic acid disappeared very rapidly upon storage. The results indicate that ascorbic acid is apparently not a factor responsible for the flavor stability of homogenized milk.

A rancid flavor and an increase in acidity were found to develop readily on storage when raw milk was mixed with homogenized pasteurized milk. The maximum increase in acidity occurred when the ratio of raw milk to homogenized pasteurized milk was approximately one to one. As the percentage of raw milk in the homogenized pasteurized milk increased above 50 per cent, the increase in titratable acidity was found to be correspondingly less. When only a small per cent of the sample was homogenized pasteurized milk very small increases in acidity occurred. These increases in titratable acidity were closely associated with the development of a rancid flavor. Dorner and Widmer (1932) were the first to note the development of rancidity when unhomogenized raw milk was mixed with homogenized pasteurized milk.

The fact that the greatest increases in acidity occurred when the milk was approximately 50 per cent raw and 50 per cent homogenized pasteurized indicates that the amount of increased surface caused by homogenization and the amount of lipase added by the raw milk are of approximately equal importance in the development of rancidity in homogenized milk. If this were true, then the increase in acidity and the development of rancidity in homogenized raw milk are dependent upon the increased surface and not upon the activation of lipase by homogenization as has been suggested.

Further evidence of the equal importance of the amount of fat surface exposed and the amount of lipase present is shown by the fact that when homogenized raw milk was added to homogenized pasteurized milk the rate of increase in acidity was only slightly greater than when unhomogenized raw milk was mixed with homogenized pasteurized milk. If lipase were activated by homogenization these increases would seem to have been considerably faster than those noted. The more rapid increase which did occur in the homogenized raw and homogenized pasteurized milk mixtures might be explained by the fact that all the fat had been subjected to homogenization so there was more fat surface exposed upon which the lipase could act than in the raw milk and homogenized pasteurized milk mixture where only a portion of the fat had been subjected to homogenization. The amount of lipase added by the raw milk seemed to be the limiting factor in the development of rancidity in homogenized raw milk. The lipase added to the homogenized pasteurized milk in the form of unhomogenized raw milk was just as effective in causing rancidity as was the lipase added by the homogenized raw milk.

SUMMARY

Pasteurized milk homogenized at 2,500 pounds pressure withstood the development of oxidized flavor over storage periods extending from 28 to 35 days at a temperature of 35° to 40° F.

The oxidation-reduction potentials of both unhomogenized and homogenized pasteurized copper-free milk showed parallel trends during storage. The addition of copper to the milk caused an increase in the oxidation-reduction potential quite similar in both the unhomogenized and in the homogenized milk.

An increase in oxidation-reduction potential was accompanied by the development of an oxidized flavor in the unhomogenized milk but not in the homogenized milk. The flavor stability of homogenized milk cannot be explained on an oxidation-reduction basis.

Unhomogenized and homogenized milk exposed to sunlight and subsequently stored showed a decrease in the oxidation-reduction potential and was accompanied by the development of an activated flavor. However, homogenized milk developed the activated flavor more quickly than did the unhomogenized milk.

The churning and washing of the fat for three times resulted in the removal of more lecithin and a more stable remade homogenized milk than those milks in which the fat had been churned and washed only one or two times.

The substance or substances affected by homogenization, thus preventing or retarding the development of oxidized flavor, were more closely associated with the cream than with the skim milk. However, some evidence was obtained indicating that some substance or substances in the

skim milk were also affected by homogenization.

Homogenization caused a slight decrease in the ascorbic acid content of fresh pasteurized copper-free milk. The ascorbic acid content of the homogenized pasteurized milk decreased more rapidly during storage than did that of the unhomogenized pasteurized or the unhomogenized raw milk. Homogenization did not have much effect upon the rate of disappearance of the ascorbic acid content of copper-treated milk during storage.

Rancidity developed readily in mixtures of milk composed of unhomogenized raw milk and homogenized pasteurized milk, with the greatest increases in acidity occurring when the ratio of unhomogenized raw milk to homogenized pasteurized milk was approximately one to one.

The development of rancidity seemed to be equally dependent upon the amount of lipase present and upon the amount of fat surface exposed by the homogenization process.

The lipase of unhomogenized raw milk when added to homogenized pasteurized milk was apparently just as effective in causing rancidity as a similar quantity of lipase in homogenized raw milk.

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