SOME STUDIES ON THE EFFECTIVENESS OF ASCORBIC ACID AND TOCOPHEROL IN RETARDING OXIDATIVE RANCIDITY IN BUTTER

by

FRANKLIN JOSEPH HEIM

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TABLE OF CONTENTS

INTRODUCTION

The deterioration of dairy products due to oxidative processes in the fat phase has been a main problem for research in dairying for many years. Carson and Thurston (2), Greenbank (9). Research in retarding or preventing this deterioration has taken several different directions, one of which is the use of antioxidants. Certain compounds have been tested for effectiveness in preventing oxidation of butterfat. Lea (16), Richardson et al. (22), and Remaly (21).

Among substances which have been found to inhibit oxidation in dry butterfat, phenolic and polyphenolic compounds are of considerable importance. The antioxidant value of ascorbic acid suspended in certain vegetable oils has been well established. Previous work by Chilson (3), who studied the effectiveness of added ascorbic acid to butter in preventing oxidative rancidity, showed that the butter developed an oxidized flavor while there was sufficient ascorbic acid present to normally retard oxidation.

When ascorbic acid is added to lard, a fat containing little or no tocopherol, its antioxidant value is considerably less than if it were added to vegetable oils containing natural tocopherols. This fact led to the postulation that ascorbic acid may require some intermediator between it and the fat since ascorbic acid, for some unknown reason, cannot supply hydrogen directly to the fat peroxides. A mechanism for this hypothesis would be:

synergistic action of ascorbic acid and tocopherol, whereas ascorbic acid is a potential reservoir of hydrogen ions for the maintenance of the stability of the fat. Tocopherol then functions to transfer hydrogen ions to the fat peroxides.

Ascorbic acid does not seem to be strongly antioxidant in the presence of very low levels of tocopherol in aqueous fat systems and since tocopherol analysis of butterfat indicates a wide variation, this may explain why certain samples develop an oxidized flavor while others seemingly are immune.

Very few antioxidants have been tested in butter, however, considerable work has been reported on ascorbic acid and tocopherol in other food fats. The special physical structure of butter makes it impossible to draw any conclusions with regard to butter from experiments on butterfat or vegetable fats.

This study was therefore undertaken to obtain additional information on effectiveness of ascorbic acid and the possible synergistic activity of ascorbic acid and d,l-alpha-tocopherol in preventing oxidative rancidity in stored butter. Since sweet, unsalted butter is the most common form of stored butterfat; therefore, the flavor of ice cream will be no better than that of the butter used in its manufacture. The problem of such oxidative off flavors as tallowy, metallic, oily, fishy, and oxidized is an important one in the dairy industry today.

REVIEW OF LITERATURE

Krukovsky et al. (14) has reported that the oxidized flavors in fresh milk are not associated with determioration of fat itself but with the fat globule membrane. He pointed out that the fat itself may undergo deterioration in the presence of ascorbic acid, resulting in the development of metallic and fishy flavors, and losses in vitamins A and E and carotenoids. According to Kummerow (15), oxidative changes in milk fats are due mainly to the presence of unsaturated fatty acids which autoxidize and produce unpalatable breakdown products. He postulates that the development of the oxidized flavor is due to a milk oxidation depending upon: 1. The percentage composition of the fatty acids and, 2. The concentrations of antioxidants and synergists.

Little is known about the offensive flavoring substances which are formed during chemical deterioration in butter according to Eilers et al. (5) who agreed with Roger's (23) idea that fishy flavors and odors of cold storage butters are caused by chemical reactions, and only when acid and oxygen are present. This deterioration was pretty generally attributed to the formation of trimethyl amine, which was though to be formed in butter by oxidation of the choline group of lecithins. Davies (4), Sommer and Smit (25).

Matti (17) states that the oily flavor in stored butter is due to oxidation of the unsaturated fatty acids released in the hydrolysis of lecithin while the fishy flavor is due to trimethyl amine (Me_3N). Supplee (26) made a study in which various amounts of trimethyl-amine were added to butter and stored. Results of his work and work by Milers et al. (5) justifies the conclusion that the defects fishy, tallowy, oily or metallic flavor cannot be caused by trimethyl amine.

That addition of copper, a mild oxidizing agent, promotes the development of the flavor has been well known but Rogers et al. (23) were the first to conclude that copper contamination caused a more intense tallowy flavor in butter than did iron. Olson and Brown (20) found that copper combines with the ascorbic acid anion and thereby promotes oxidation. As ascorbic acid was one of the antioxidants worked with in this study this fact should be considered.

The chemical properties of the milk which have been considered as affecting the development of an oxidized flavor are Eh, pH, and the concentration of antioxidants either inherent or added especially for this purpose.

Matti (17) reported that the formation of fishy and oily flavors in butter can be eliminated almost entirely by raising the pH of the butter to 6-7 by addition of a mixture of NaCl, Na_2HPO_4 and Na_2Co_3. The neutralization of butter above pH 6 does not appear to be a sufficient guarantee against oxidation,

he continues, the latter apparently is related to a shift in the oxidation-reduction potential from minus to plus. Definite relationships exist between the pH value of butter and its keeping quality, according to Munin (18). Low pH values, he says, indicate increased peroxide formation and low quality butter; butter having a pH value below 6 tends to become tallowy and should not be stored.

Matti (17) states that the addition of NaCl retards oxidation in some manner which he postulates to be related to the oxidation-reduction potential of butter. Tracing the oxidation by the Lea fat-peroxide test and the Sehibsted fat-aldehyde test he concluded that the chloride ions, released by reaction between fat-hydroperoxide and hydrogen and chloride ions, activates further oxidation.

Saal (24) studied factors influencing the keeping quality of butter by measuring the oxidation-reduction potential in order to determine any connection between taste and oxidative processes. He found that oxidizing constituents present gave the plasma a higher potential and reducing agents such as ascorbic acid or lactoflavin gave it a lower potential than distilled water at the same pH and oxygen content. The ascorbic acid in the plasma of butter disappears shortly after the manufacture of butter and considerable quantities of oxidizing agents are formed increasing the potential. He failed, however, to measure the concentration of oxidizing agents and the tendency of the butter to

develop undesirable flavors by the oxidation-reduction potential. Eilers et al. (5) reported that the potential rises quickly in butter whereas milk exhibits a slow rise in potential and thus enables the degree of oxidation to be gauged. On account of this it is not possible to use the change in potential as a measure of the oxidative rancidity in butter, he concluded.

Tracy, Ramsey, and Reuhe (27) found that the addition of copper caused an increase in the Eh of susceptible milks. This conclusion was verified by Thurston (28) and Webb and Hileman (30). Greenbank (9) concluded that the inhibition of the flavor by bacterial growth and by heat has been attributed to the lowering of the Eh. Hartman, Garrett, and Button (10) found that an addition of copper ($CuSO_4$) to milk caused an increase in the O-R potential, the speed of increase and the magnitude reached depending upon the concentration of copper. With higher concentrations of copper the maximum potential was reached in a relatively short time and this level maintained throughout the period.

Greenbank (9) also states that the use of poor feed increases the Eh and promotes the development of oxidized flavor in milk while green feed lowers the Eh and development of the flavor is inhibited.

Thurston (29) classified milks as 1. spontaneous -- those which develop an oxidized flavor without the addition of copper, 2. susceptible -- those which develop the flavor with the addition of a small amount of copper, and 3. non-susceptible --

those which do not develop an oxidized flavor even if copper is added.

Webb and Hileman (30) were able to predict with a fair degree of accuracy the susceptibility of samples by the rise of Eh after the addition of copper.

The resistance of milk to a change in Eh; analogous to buffering in the acid-base system is called poising. Greenbank (7) concluded that the variations in samples is a result of differences in poising. When poising is used as a criterion, according to him, spontaneous milks are those which are very poorly poised, susceptible milks those poorly poised and non-susceptible milks those which are well poised.

Hartman, Garrett and Button (10) concluded that the addition of synthetic crystalline ascorbic acids greatly decreased the potential, the amount of decrease proportional to the concentration added. The same workers found that additions of both soluable copper and synthetic ascorbic acid to milk caused a rise in potential the magnitude and rate being dependent upon the concentration of copper and acid added.

Krukovsky et al. (13) found that ascorbic acid plays an important part in the oxidative deterioration of milk fat at the end of its storage life resulting in the development of objectionable flavors and losses in vitamins A and E and the carotene content of the fat. The susceptibility of fat to this type of deterioration is determined primarily by the treatment of the

milk, the temperature of pasteurization, the type of product, the condition of storage, and to a lesser extent upon the direct and immediate effect of the exposure to light.

The ratio of the reduced form to the oxidized form of ascorbic acid, according to Greenbank (9), may be reflected by the Eh of the milk because the ascorbicdehydroascorbic acid system is reversible.

Olson and Brown (19) postulated the mechanism for the development of oxidized flavor in milk might be the oxidation of ascorbic acid with the production of H_2O_2 which then oxidizes the phospholipids liberating the compounds which give the oxidized flavor.

Krukovsky and Guthrie (11) also concluded that ascorbic acid is a link in the chain forming the flavor. They based this conclusion on the observation that the oxidation of ascorbic acid by H_2O_2 inhibits development and milk so treated can be made to develop the flavor by adding ascorbic acid. Later the same authors (12) concluded it is the ascorbic-dehydroascorbic acid ratio which controls the development of the flavor.

Greenbank (8) explains these reactions according to his intermediate oxidation product theory, which concludes that when all the ascorbic acid is destroyed the Eh is high enough to produce a completely oxidized form which has no flavor. The addition of more ascorbic acid to the milk lowers the Eh so that the intermediate or flavored compound may form.

A significant correlation (≠0.51) was found by Krukovsky, loosli and Whiting (14) between the tocopherol content of milk fat and the ability of milk to resist the reaction, involving ascorbic acid oxidation, which produces oxidized flavors. This might explain the differences between the stabilities of winter and summer milks, they concluded.

Recently Godel (6) reported positive results concerning vitamin E as a method of controlling the development of a bitter taste in butter.

It is probable that the bitter flavor and one involving oxidative rancidity may be dissimilar. He reported that additions of vitamin E in the form of wheat or corn oil, at the level of 0.2-2.0 per cent milk fat gave proportionally higher stability to oxidation in air; similar protection was given to butter in storage up to 120 days at 5° C. and 40 days at 20-25° C.

Munin (18) has recently reported on Danish investigations involving the addition of fat rich in hydroxyl groups (wheat germ oil) to butter in an attempt to counteract rancidity. No conclusions of this study were made.

EXPERIMENTAL PROCEDURE

It was planned to study the development of an oxidized flavor in the fresh and stored butter by organoleptic tests and possibly find some correlation between the flavors occurrence and a change in the pH, Eh, and ascorbic acid content of the butter sera. Ascorbic acid and d,l-alpha-tocopherol were the antioxidants used in the study herein reported.

Preparation of Butter Samples

Three commercial size churnings were made, two at the Kansas State College Creamery and one at a local creamery. The first churning was made from sour cream and produced 91 score butter. The second churning was made from sweet cream separated from milk from the college herd and churned into 92 score butter. The third churning was made from sour, station cream, and produced 90.5 score butter.

A 12 pound aliquot of butter was taken from the churn just after the butter was washed and before salt was added. A moisture test was run and 12 portions were weighed out to contain 352 grams of fat each. To each portion was added 10 grams of butter oil or 10 grams of butter oil containing 0.01 gram of d,l-alpha-tocopherol. To certain portions a solution of ascorbic acid (0.1 gram per 163 grams of water) or a solution of ascorbic acid (0.01 gram per 163 grams of water) was added. A solution of copper (0.5 ppm. as copper sulfate) was added to certain portions to accentuate the oxidized flavor. Enough water was then worked into each sample to produce one pound of butter. The butter was then weighed, chemicals were added and was worked in a constant temperature room set at 50° F. to insure proper temperature control in producing butter with a good texture.

Treatment of Individual Samples

Sample 1 contained 352 grams of butter and 10 grams of butter oil and was held as the control.

Sample 2 contained 352 grams of butter, 10 grams of butter oil and 0.1 gram of ascorbic acid per 163 grams of water.

Sample 3 contained 352 grams of butter plus 10 grams of butter oil containing 0.01 gram of d,l-alpha-tocopherol.

Sample 4 contained 352 grams of butter, 10 grams of butter oil containing 0.01 gram of tocopherol and 0.1 gram of ascorbic acid per 163 grams of water.

Samples 5, 6, 7, and 8 were the same as 1, 2, 3, and 4 respectively with the exception that to each was added a solution of copper (0.5 ppm. as copper sulfate).

Sample 9 contained 352 grams of butter, 10 grams of butter oil and 0.01 gram of ascorbic acid per 163 grams of water.

Sample 10 contained 352 grams of butter, 10 grams of butter oil containing 0.01 gram of tocopherol and 0.01 gram of ascorbic acid per 163 grams of water.

Samples 11 and 12 were the same as 9 and 10 respectively with the exception that to each was added a solution of copper (0.5 ppm. as cooper sulfate).

Each of these one pound samples was then divided into four parts and stored in 4 ounce, screw top jars, two at 40° F. and two in the ice cream hardening room at -10° F.

Individual Churnings

Later two additional churnings were made when oxidized flavor appeared in the market milk. They were designed to study the appearance of an oxidized flavor in butter churned from

spontaneously and non-spontaneously oxidized samples of milk. Samples of each cow in the college herd (85-90 head) were taken into clean half pint bottles, pasteurized at 145° F. for 30 minutes and stored for three days at 40° F. They were then scored by two judges independently for an oxidized flavor each day for five days.

Three cows (two Holsteins and one Jersey) were then selected to continue flavor tests and were classified as producing milk which developed an oxidized flavor spontaneously. Three other cows (two Holsteins and one Jersey) were also selected because the flavor tests showed they produced milk which did not spontaneously develop an oxidized flavor. At least six flavor tests on the fluid milk were run to classify each cow.

The complete milking of each cow was collected in a clean, stainless steel pail, promptly pasteurized at 145° F. for 30 minutes, cooled to 40° F. rapidly and stored at that temperature. This process was repeated for three days until enough milk was collected to churn. It was then separated into 33 per cent cream and pooled together as either spontaneous or non-spontaneous. Using a Dazey experimental churn the cream was churned into butter in approximately 45 minutes at 50° F.

Treatment of Butter from Individual Churnings

Eight 352 gram portions of the butter were weighed out and worked by hand to the proper composition and texture. To each portion 10 grams of butter oil or 10 grams of butter oil containing

0.01 gram of d,l-alpha-tocopherol was added. To certain portions 0.1 gram of ascorbic acid was added to the cream before churning. The concentration was thus .016 gram per lb. of butter. Enough water was then worked into the sample to produce one pound of butter with commercial composition and texture.

Butter Churned from Spontaneously Oxidized Milk

Sample 1 contained 352 grams of butter and 10 grams of butter oil.

Sample 2 contained 352 grams of butter, 10 grams of butter oil, and .016 gram of ascorbic acid.

Sample 3 contained 352 grams of butter and 10 grams of butter oil containing 0.01 gram of tocopherol.

Sample 4 contained 352 grams of butter, 10 grams of butter oil containing 0.01 gram of tocopherol and .016 gram of ascorbic acid.

Butter Churned from Non-Spontaneous Milk

Samples 1, 2, 3, and 4 in this classification were treated identically as 1, 2, 3, and 4 above although made from non-spontaneously oxidized milk.

Each of these one pound samples was then divided into four parts and stored in 4 ounce jars, two at 40° F. and two in the hardening room at -10° F.

At the time these churnings were made the cows were permanently off pasture and no copper was added to develop the flavor.

Flavor Scores on Stored Butter

Each butter sample was tempered at about 60° F. and was examined organoleptically by two experienced judges working independently. Flavor scores were recorded of the fresh butter, after three weeks at 40° F., after eight weeks at 40° F., after five months at -10° F. and after six months at -10° F. The scores were then averaged and put into tabular form. Scoring was done according to the standard procedure for dairy products with a perfect flavor score of 45 points. The intensity of the oxidized flavor was indicated by the drop in score of the sample. All other off-flavors were recorded, but were not listed on the tables.

pH and Eh Values on Butter Sera

The 4 ounce samples were tempered to 122° F. in a water bath until the butterfat and serum separated. A nine ml sample of sera was then taken and pH and Eh measurements were made using a Leeds-Northrup potentiometer. pH values were obtained using a glass indicating electrode and a saturated calomel reference electrode. Eh measurements were with a platinum indicating electrode and a calomel reference. These measurements were taken on the fresh samples, after storage for three and eight weeks at 40° F. and after five and six months storage at -10° F.

Ascorbic Acid Determinations

Ascorbic acid determinations were made according to the method developed by Bessey and King (1), and modified by Woessner, Elvehjem and Schuette (31).

In these tests 2 ml of sera stood 30 minutes with 6 ml of Willburg reagent. Two or five ml aliquots were titrated with 2-6 dichloro-phenol-indophenol to a faint pink end point. The results were expressed as milligrams of ascorbic acid per liter of serum (ppm.). Tests were made of all samples of fresh and stored butter.

PRESENTATION OF EXPERIMENTAL RESULTS

Table 1 presents the average of three trials of flavor scores with different samples of butter treated with ascorbic acid and c,l-alpha-tocopherol when fresh and after three and eight weeks storage at 40° F. Three of the samples which dropped the most in score (2.9 points) during storage contained ascorbic acid, ascorbic acid and tocopherol and tocopherol plus copper. The change in score of the control was 2.2 points. All samples with a larger drop in score than the control had an oxidized flavor criticism while none of the samples whose change in score was equal or less than the control developed the flavor. In four out of six samples which dropped in score more than the control the oxidized flavor was detected after three weeks storage. Ascorbic acid had been added to five of these samples and tocopherol

Table 1. Effect of ascorbic acid and d,l-alpha tocopherol in retarding the development of an oxidized flavor in butter stored at 40° F.

Treatment	Flavor scores (av. of two judges)							
	Fresh butter		3 wks. stored at 40° F.			8 wks. stored at 40° F.		
	Average*	Range	Average*	Range	Av. change	Average*	Range	Av. change
Control	91.2	90.5-92.0	90.4	89.5-91.0	- .8	89.0	88.5-91.0	-2.2
Control + ascorbic acid (1)	91.5	90.5-92.0	≠89.5	88. -90.5	-2.0	≠88.6	88. -89.2	-2.9
Control + tocopherol	91.2	90.5-92.0	90.1	88.5-91.0	-1.1	89.2	88.2-90.0	-2.0
Control + ascorbic acid (1) + tocopherol	91.2	90.4-92.0	≠89.1	88. -90.5	-2.1	≠88.3	87.5-90.5	-2.9
Control + copper	91.2	90.5-92.0	90.5	88. -91.25	- .7	88.7	88. -89.8	-2.5
Control + ascorbic acid (1) + copper	91.2	90.5-92.0	89.3	88. -90.5	-1.9	≠88.6	87. -90.5	-2.6
Control + tocopherol + copper	91.2	90.5-92.0	≠89.0	88. -90.	-1.6	≠88.3	87.2-90.0	-2.9
Control + ascorbic acid (1) + tocopherol + copper	91.2	90.5-92.0	90.2	88. -91.25	-1.0	≠88.0	86. -89.5	-2.8
Control + ascorbic acid (2)	91.2	90.5-92.0	90.2	90.5-92.0	-1.0	89.0	88. -90.5	-2.2
Control + ascorbic acid (2) + tocopherol	91.2	90.5-92.0	≠89.9	90.5-92.0	-1.3	≠88.8	88. -90.0	-2.4
Control + ascorbic acid (2) + copper	91.2	90.5-92.0	90.0	90.5-92.0	-1.2	89.1	88. -90.1	-2.1
Control + ascorbic acid (2) + tocopherol + copper	91.2	90.5-92.0	90.2	90.5-92.0	-1.0	89.4	88. -90.2	-1.8

Control - Commercial butter
Ascorbic acid (1) - 0.10 gms per 163 gms water
Ascorbic acid (2) - 0.01 gms per 163 gms water
Tocopherol - .01 gm d,l-alpha-tocopherol per lb. butter
Copper - 0.5 ppm. $CuSO_4$
*Average of three trials
≠Oxidized flavor criticism

alone to one. The addition of tocopherol improved that sample as it only dropped 2.0 points while the same containing tocopherol plus copper dropped 2.9 points. No synergistic relationship was found when ascorbic acid and tocopherol were added the flavor dropping 2.9 points as compared to a 2.2 point drop in the control. Of the three samples whose score dropped less than the control, the least drop (1.8 points) occurred when the sample was treated with a lower concentration of ascorbic acid, tocopherol and copper. Of the other two samples which dropped less than the control, tocopherol additions held the drop in score to 2.0 points and a lower concentration of ascorbic acid plus copper produced a 2.1 point drop in score. These three samples were not criticized for an oxidized flavor; the treatments apparently minimmized other off flavors.

Table 2 presents the changes in flavor score of butter samples with ascorbic acid and tocopherol added after storage for six months at -10° F. A large majority (10) of the samples developed an oxidized flavor after six months storage while only two were criticized for this flavor after five months storage. One of the above two samples was treated with a low concentration of ascorbic acid and had a flavor score drop of 0.7 point compared to the drop in the control of 1.2 points. The other sample contained tocopherol and only dropped 0.7 point after six months' storage. The four samples whose flavor score dropped more than the control contained different levels of ascorbic acid plus tocopherol. There appears therefore, that after six

Table 2. The effect of ascorbic acid and d,l-alpha tocopherol in retarding the development of an oxidized flavor in butter stored at -10° F.

Treatment	Flavor scores (av. of two judges)							
	Fresh butter		3 mo. stored at -10° F.			6 mo. stored at -10° F.		
	Average*	Range	Average*	Range	Av. change	Average	Range	Av. change
Control	91.2	90.5-92.0	90.5	90.0-91.0	-0.7	≠90.0	89.0-91.0	-1.2
Control + ascorbic acid (1)	91.5	90.5-92.0	≠89.5	88.0-90.1	-2.0	90.5	90.0-91.0	-1.0
Control + tocopherol	91.2	90.5-92.0	90.0	88.5-90.5	-1.2	≠90.5	90.0-91.0	-0.7
Control + ascorbic acid (1) + tocopherol	91.2	90.5-92.0	90.0	89.0-91.0	-1.2	≠90.6	90.0-91.5	-0.7
Control + copper	91.2	90.5-92.0	90.0	88.5-91.0	-1.2	≠89.5	89.0-90.0	-1.7
Control + ascorbic acid (1) + copper	91.2	90.5-92.0	≠89.8	89.5-90.0	-1.4	≠89.5	89.0-90.5	-1.7
Control + tocopherol + copper	91.2	90.5-92.0	90.8	89.0-91.0	-0.4	≠90.0	89.6-91.0	-1.2
Control + ascorbic acid (1) + tocopherol + copper	91.2	90.5-92.0	≠88.5	88.0-89.0	-2.7	≠90.0	89.0-91.0	-1.2
Control + ascorbic acid (2)	91.2	90.5-92.0	90.5	90.0-91.0	-0.7	≠90.5	90.0-91.0	-0.7
Control + ascorbic acid (2) + tocopherol	91.2	90.5-92.0	≠88.8	88.0-89.5	-2.4	90.5	90.0-91.0	-0.7
Control + ascorbic acid (2) + copper	91.2	90.5-92.0	90.0	89.0-91.0	-1.2	≠89.3	89.0-89.5	-1.9
Control + ascorbic acid (2) + tocopherol + copper	91.2	90.5-92.0	90.0	89.0-91.0	-1.2	≠89.7	89.5-90.0	-1.5

Control - commercial butter
Ascorbic acid (1) - 0.10 gms per 163 gms water
Ascorbic acid (2) - 0.01 gms per 163 gms water
Tocopherol - 0.01 gm. d,l-alpha tocopherol per lb. butter
Copper - 0.5 ppm. $CuSO_4$
*Average of three trials
≠ Oxidized flavor criticism

months' storage at -10° F., some protection against off flavors was offered by the ascorbic acid and tocopherol additions.

Table 3 presents the average of three trials of pH, Eh and ascorbic acid changes during three weeks of eight weeks storage at 40° F. It can be noticed from this table that samples to which ascorbic acid had been added contained more ascorbic acid at the end of the storage period. This was true even if copper, a milk oxidizing agent, had been added. The four samples to which a small concentration of ascorbic acid was added had higher final ascorbic acid values when tocopherol was present than without this synergist. The reverse was true if higher concentrations of ascorbic acid was added with tocopherol. Five out of the six samples with added ascorbic acid developed the oxidized flavor the final ascorbic acid concentrations of these samples were 1.9 to 2.9 times the control after eight weeks' storage. The one sample which developed an oxidized flavor without ascorbic acid contained added tocopherol and copper. The ascorbic acid decrease in this sample was 10.0 mg as compared to only a 3.7 mg drop in the control. It is evident from Table 3, therefore, that additions of tocopherol lowered the ascorbic acid value most severely, but without the appearance of an oxidized flavor.

The data in Table 3 also show the continuous decrease in pH of all samples as the storage period was extended to eight weeks at 40° F. After six weeks' storage some drop in pH was

Table 3. Effect of adding ascorbic acid and tocopherol to butter on the changes in pH, Eh, and the vitamin C content during storage at 40° F.

Treatment	Fresh*			3 wks. stored at 40° F.*					8 wks. stored at 40° F.*					
	Av. pH	Av. Eh Mv	Av. vit. C (ppm)	Av. pH	pH change	Av. Eh	Eh change	Av. vit. C (ppm)	Vit. C. change	Av. pH	pH change	Av. Eh	Eh change	Av. vit. C. (ppm)	Vit. C. change
Control	6.97	233	6.9	6.35	- .62	250	+ 17	5.6	- 1.3	5.90	-1.07	258	+ 25	3.2	- 3.7
Control+ascorbic acid (1)	7.21	94	80.6	6.18	-1.03	210	+116	21.5	-59.1	5.60	-1.61	215	+121	9.2	-71.4
Control+tocopherol	7.25	184	7.9	6.45	- .80	230	+ 46	6.8	- 1.1	5.75	-1.50	80	-104	1.9	- 6.0
Control+ascorbic acid (1)+tocopherol	6.92	97	78.4	6.08	- .84	205	+108	24.3	-54.1	5.95	-1.57	140	+ 43	6.3	-72.1
Control+copper	7.16	174	9.6	6.32	- .84	220	+ 46	7.9	- 1.7	5.70	-1.46	150	- 24	3.2	- 6.4
Control+ascorbic acid (1)+copper	6.94	114	75.5	6.10	- .84	190	+ 76	23.5	-52.0	5.40	-1.54	150	+ 36	5.2	-70.3
Control+tocopherol+copper	6.98	170	12.3	6.40	- .58	160	-10	10.2	- 2.1	5.85	-1.13	160	- 10	2.3	-10.0
Control+ascorbic acid (1)+tocopherol+copper	6.86	112	68.5	6.10	- .76	190	+78	18.8	-49.7	5.70	-1.16	162	+ 50	5.0	-63.5
Control+ascorbic acid (2)	6.98	140	21.5	6.45	- .53	90	-50	12.8	- 8.7	5.75	-1.23	170	+ 30	4.7	-16.8
Control+ascorbic acid (2)+tocopherol	7.03	175	24.8	6.55	- .48	210	+35	14.3	-10.5	5.80	-1.23	156	- 19	5.4	-19.4
Control+ascorbic acid (2)+copper	7.00	181	18.8	6.45	- .55	200	+19	6.0	-12.8	5.90	-1.10	110	- 71	3.2	-15.6
Control+ascorbic acid (2)+tocopherol+copper	7.00	192	25.4	6.45	- .55	215	+23	8.6	-16.8	5.90	-1.10	160	- 32	41	-21.3

Control - commercial butter
Ascorbic acid (1) - 0.10 gms per 163 gms water
Ascorbic acid (2) - 0.01 gms per 163 gms water
Tocopherol - .01 gm d,l-alpha tocopherol per lb. butter
Copper - 0.5 ppm $CuSO_4$
*Average of three trials

noticed as six samples dropped more than the control. The largest drop in pH was exhibited by the sample containing ascorbic acid (1.03 points while the control dropped .62 point) although its initial pH was very high (7.21). The next largest pH drops were the sample containing copper only (1.46 points, the one containing ascorbic acid plus copper (1.54 points), and sample 4 containing ascorbic acid and tocopherol (1.57). The drop in pH of the control after eight weeks storage at 40° F. was 1.07 points. The samples whose pH changed least contained a lower concentration of ascorbic acid and tocopherol (.48 point) and the next smallest change of .53 point was produced by the sample containing a lower concentration of ascorbic acid. The control exhibited a pH decrease of .62 point after three weeks' storage at 40° F. Although the data in Table 3 show the total change in pH after eight weeks storage, comparisons of the pH during the first three weeks and the last five weeks show more distinctive differences. Thus, as the data in Table 3 show, no single treatment or one combination of treatments produced systematic pH changes consistently.

In discussing the Eh of the samples Tables 3 and 4 are presented to show relationships between the Eh and treatments the samples received. The Eh of the control was the highest initial Eh (233 mv) and this sample continued to be highest in Eh during the storage periods. This was rather surprising since the addition of copper, a mild oxidizing agent, should have increased

Table 4. Effect of adding ascorbic acid and tocopherol to butter on the changes in pH, Eh, and the Vitamin C content during storage at -10° F.

Treatment	Fresh*			5 mos. stored at -10° F.*					6 mos. stored at -10° F.*					
	Av. pH	Av. Eh mv	Av. vit. C (ppm)	Av. pH	pH change	Av. Eh	Eh change	Av. vit. C (ppm)	Vit. C. change	Av. pH	pH change	Av. Eh	Eh change	Av. vit. C (ppm)	Vit. C. change
Control	6.97	233	6.9	6.40	-.57	25	+17	7.0	+ 0.1	7.30	+0.33	197	-36	7.4	+ 0.5
Control+ascorbic acid (1)	7.21	94	80.6	6.07	-1.14	133	+39	46.5	-34.1	7.34	+0.13	122	+28	34.8	-45.8
Control+tocopherol	7.25	184	7.9	6.70	-.55	183	- 1	6.3	- 1.6	7.62	+0.37	159	-25	5.8	- 2.1
Control+ascorbic acid (1)+tocopherol	6.92	97	78.4	6.72	-.20	137	+40	52.6	-25.8	7.40	+0.48	111	+14	50.6	-27.2
Control+copper	7.16	174	7.9	6.91	-.25	175	+ 1	8.6	+ 0.7	7.40	+0.24	145	-29	5.7	- 2.2
Control+ascorbic acid (1)+copper	6.94	114	75.5	6.80	-.14	145	+31	62.6	-12.9	7.32	+0.38	134	+20	52.6	-22.9
Control+tocopherol+copper	6.98	17	12.3	6.82	-.16	18	+10	11.6	- 0.7	7.45	+0.47	155	-15	8.6	-22.9
Control+ascorbic acid (1)+tocopherol+copper	6.86	112	68.5	6.72	-.14	17	+58	60.3	-08.2	7.30	+0.44	145	+33	47.3	-21.2
Control+ascorbic acid (2)	6.98	14	21.5	6.88	-.10	188	+48	11.9	-09.6	7.41	+0.43	163	+23	12.2	-09.3
Control+ascorbic acid (2)+tocopherol	7.03	175	24.8	7.02	-.01	192	+17	17.9	-06.9	7.13	+0.10	200	+25	8.4	-16.4
Control+ascorbic acid (2)+copper	7.00	181	18.8	6.93	-.07	215	+34	14.0	-04.8	6.80	-0.20	195	+14	8.4	-10.4
Control+ascorbic acid (2)+tocopherol+copper	7.00	192	25.4	7.00	-	194	+ 2	16.0	-09.4	7.20	+0.20	172	-20	9.6	-15.8

Control - commercial butter
Ascorbic acid (1) - 0.10 gms per 163 gms water
Ascorbic acid (2) - 0.01 gms per 163 gms water
Tocopherol - 0.01 gm d,l-tocopherol per lb. butter
Copper - 0.5 ppm $CuSO_4$
*Average of three trials

instead of decreasing the samples Eh (174 mv). In the fresh butter the four samples containing ascorbic acid had the lowest Eh values even if copper or tocopherol or both were also added. The sample treated with tocopherol showed a higher initial Eh value (184 mv) than the sample containing copper (174 mv). The sample containing a lower concentration of ascorbic acid and copper also had a higher initial Eh (181 mv) than the sample containing copper. The two lowest Eh values were among the first to show an oxidized flavor after storage at 40° F. and at -10° F. These samples contained ascorbic acid and ascorbic acid plus tocopherol and had initial Eh values of 94 and 97 mv respectively.

Of the Eh changes during storage at 40° F., the first noticed was an increase in Eh of ten of the twelve samples after three weeks storage. The two samples which decreased in Eh were those containing tocopherol plus copper and that one containing a lower concentration of ascorbic acid. The four samples (containing ascorbic acid) with the lowest Eh values when fresh showed the largest increase after three weeks of storage. The three smallest increases in Eh were those samples containing a lower concentration of ascorbic acid plus tocopherol and copper, or tocopherol or copper alone. Although the lower concentration of ascorbic acid did not decrease the Eh as much as the higher concentration of ascorbic acid did not decrease the Eh as much as the higher concentration of ascorbic acid in the fresh butter and after three weeks storage, the sample with the lower concentration of ascorbic acid had a slightly lower Eh (90 mv) while

the sample containing a higher concentration of ascorbic acid exhibited a maximum increase during storage (210 mv).

After eight weeks storage, only three samples had higher Eh values than after three weeks storage. These samples contained a higher concentration of ascorbic acid, a lower concentration of ascorbic acid and the control. Eh values were 215, 170, and 258 mv, respectively. Of the remaining seven samples showing a decrease or smaller increase in Eh, four contained tocopherol. These four decreases, however, were from very high initial values (184, 170, 175, and 192 mv). The two largest Eh increases accompanied the development of an oxidized flavor while the other two oxidized flavors reported were accompanied by a small increase and an actual decrease.

Thus it is apparent that the oxidized flavor did not consistently accompany either a large or small increase in Eh. The two samples showing an oxidized flavor after eight weeks storage but not after three weeks both decreased in Eh during the last five weeks although the final Eh proved to be higher than the initial. Therefore, the oxidized flavor commonly considered to be due to a high Eh value did not appear until the initial rise in Eh had passed its maximum.

The data in Table 4 presents the average of three trials of different samples of butter treated when fresh and after five and six months storage at -10° F. The six samples whose Eh increased most after storage for five months, developed an oxidized flavor either then or after six months storage. One sample, containing

ascorbic acid, had an oxidized flavor criticism at five months but after six months storage seemed to have improved in flavor. The flavor scores in this sample were accompanied by a moderate Eh increase at five months and an additional rise after six months of storage. Five other samples developed an oxidized flavor after six months storage and all exhibited small increases in Eh up to five months storage followed by a decrease.

Table 5 presents the changes in flavor score of samples with additions of ascorbic acid and tocopherol after storage at 40° F. and at -10° F. The table consists of two parts: The butter churned from spontaneously oxidized and that churned from non-spontaneously oxidized milk. After six weeks storage, flavor scores and criticism indicate that the only oxidized flavor found was the sample of spontaneous butter treated with ascorbic acid. Thus tocopherol, in this case, inhibited the detrimental effect of the ascorbic acid but was not shown to improve the control. All samples from spontaneously oxidized milk and none of the samples from non-spontaneously oxidized milk had developed oxidized flavor after three months storage regarless of the treatment. After four months storage the non-spontaneous samples were oxidized if treated with tocopherol alone or tocopherol plus ascorbic acid.

The data in Table 6 present the changes in pH, Eh, and ascorbic acid content after treatment of the samples treated with ascorbic acid and tocopherol after three and six weeks storage at 40° F. This table and Table 7 which presents the same chemical changes after storage for three and four months at -10° F.

Table 5. Effect of additions of ascorbic acid and tocopherol in retarding the development of an oxidized flavor in butter churned from spontaneous and non-spontaneous oxidizing milk.

Treatment	Flavor scores (Av. of two judges)										
	3 wks. stored and 6 wks. stored at 40° F.					3 mos. stored at -10° F.			4 mos. stored at -10° F.		
	Av.* score	Range	Av.* score	Range	Av. change	Av.* score	Range	Av. change	Av.* score	Range	Av. change
Butter churned from samples of spontaneously oxidized milk											
Control butter	92	-	91.25	90.5-92.0	- .75	≠91.0	-	- .1	≠91.5	91.0-92.0	- .5
Control+ascorbic acid	91.5	91-92	≠90.25	90.0-90.5	-1.25	≠91.25	91.0-91.5	- .25	≠91.0	90.0 92.0	- .5
Control+tocopherol	91.0	90-92	91.0	90-92	-	≠91.5	91-92	- .5	≠91.5	-	+ .5
Control+ascorbic acid + tocopherol	91.5	91-92	91.87	91.75-92	+.32	≠91.5	91-92	-	≠90.6	90.25-91.0	+ .1
Butter churned from samples of non-spontaneously oxidized milk											
Control	91.5	91-92	91.25	90.5-92.0	- .25	91.75	91.5-92.0	- .25	92.25	91.5-93	+ .75
Control-ascorbic acid	91.0	90-92	91.25	90.5-92.0	+ .25	91.75	91.5-92.0	- .75	92.0	91-93	+1.0
Control+tocopherol	91.0	90-92	91.5	91.0-92.0	+ .5	91.75	91.5-92.0	- .75	≠91.75	90.5-93	+ .75
Control+ascorbic acid +tocopherol	91.5	91-92	91.25	90.5-92.0	- .25	91.75	91.5-92.0	- .25	≠91.0	90.5-91.25	+ .5

Control - hand churned sweet cream butter
Ascorbic acid - 21 mg per 100 ml serum
Tocopherol - 1 gm d,l tocopherol per lb. butter

*Average of two trials
≠oxidized flavor criticism

Table 6. The effect of added ascorbic acid and d,l-alpha-tocopherol on the change in pH, Eh, and the Vitamin C content of butter churned from spontaneous and non-spontaneous milk.

Treatment	3 wks. stored at 40° F.			6 wks. stored at 40° F.				
	pH*	Eh*	Vit.C.*(ppm)	pH*	Change in pH	Eh*	Vit. C* (ppm)	Change in vit. C
Butter churned from samples of spontaneously oxidized milk								
Control butter	6.40	200	21.4	6.54	.14	158	21.5	0.1
Control ascorbic acid	6.25	210	28.6	≠6.60	.35	≠154	≠28.0	-0.6
Control tocopherol	6.35	210	21.4	6.73	.38	218	20.0	-1.4
Control ascorbic acid tocopherol	6.35	210	25.0	6.63	.40	200	26.0	-1.0
Butter churned from samples of non-spontaneously oxidized milk								
Control butter	6.30	190	23.8	6.76	.46	210	16.2	-7.6
Control ascorbic acid	6.01	180	29.7	6.86	.85	166	26.8	-2.9
Control tocopherol	6.25	185	26.2	6.41	.16	156	19.3	-6.9
Control ascorbic acid tocopherol	6.25	190	33.3	6.66	.41	148	30.8	-2.5

Control - hand churned sweet cream butter
Ascorbic acid - 21 mg per 100 ml serum
Tocopherol - 0.1 gm d,l tocopherol per lb. butter
*Average of two trials
≠Oxidized flavor criticism

Table 7. The effect of added ascorbic acid and d,l alpha-tocopherol on the change in pH, Eh, and the vitamin C content of butter churned from spontaneous and non-spontaneous milk

Treatment	3 mos. stored at -10° F.					4 mos. stored at -10° F.				
	pH*	Change in pH	Eh*	Vit. C ppm*	Change in vit.C	pH*	Change in pH	Eh*	Vit. C ppm*	Change in vit.C
Butter churned from samples of spontaneously oxidized milk										
Control butter	≠7.16	.76	≠154	≠12.9	- 8.5	≠6.00	-.40	≠.146	≠ 7.5	-13.9
Control ascorbic acid	≠6.57	.32	≠103	≠15.0	-13.6	≠6.30	.05	≠.138	≠ 9.3	-19.3
Control tocopherol	≠7.16	.81	≠150	≠12.5	- 8.9	≠6.08	-.27	≠.153	≠ 9.4	-12.0
Control ascorbic acid tocopherol	≠6.70	.47	≠148	≠18.3	- 6.7	≠6.50	.27	≠.139	≠11.5	-13.5
Butter churned from samples of non-spontaneously oxidized milk										
Control butter	7.13	1.01	171	11.0	-12.8	6.73	.43	.155	10.0	-13.8
Control ascorbic acid	7.12	1.11	146	28.6	- 1.1	≠6.42	.41	≠.120	≠16.9	-12.8
Control tocopherol	7.16	.91	151	13.2	-13.0	≠6.33	.08	≠.135	≠ 7.5	-18.7
Control ascorbic acid tocopherol	7.15	.90	140	31.2	- 2.1	≠6.33	.08	≠.120	≠17.6	-15.7

Control - hand churned sweet cream butter
Ascorbic acid - 21. mg per 100 ml serum
Tocopherol - 0.1 gm d,l tocopherol per lb. butter
*Average of two trials
≠Oxidized flavor criticism

In all cases a considerable portion of the ascorbic acid remained at the end of the experiment at either 40° F. or -10° F. Considering this fact it is evident that the failure of ascorbic acid to prevent the development of an oxidized flavor cannot be ascribed to its disappearance. Further examination of the data in Tables 6 and 7 showed that in seven out of eight samples ascorbic acid lowered the pH of the samples both after storage for three weeks at 40° F. or three months at -10° F. This effect, however, had reversed after storage for either six weeks storage at 40° F. or after four months at -10° F. These changes in pH were not large and probably not significant in relation to the oxidized flavor criticism.

There is some tendency for the Eh of the butter after shorter storage periods to be lowered by the additions of ascorbic acid and raised by the additions of tocopherol. This relationship was not found after storage for periods exceeding two months.

SUMMARY

1. None of the samples whose change in score was equal to or less than the control developed an oxidized flavor. Addition of copper was the only treatment which produced a larger drop in flavor score without producing an oxidized flavor.

2. In the 3 samples with the least drop in flavor score treatment with ascorbic acid and tocopherol apparently minimized other off flavors besides an oxidized flavor.

3. The 4 samples whose flavor score dropped more than the control in 6 months storage at -10° F. contained varying amounts of added ascorbic acid and copper.

4. The final ascorbic acid content of samples with a small amount of ascorbic acid added was higher after 8 weeks storage at 40° F. when tocopherol was present. With no added ascorbic acid or with a larger addition the final ascorbic acid values were lower when tocopherol was present.

5. Five out of the six samples which had ascorbic acid added developed the oxidized flavor after 8 weeks storage at 40° F. The final ascorbic acid concentrations were from 1.9 to 2.9 times the concentration of ascorbic acid in the control. Thus the failure of ascorbic acid to prevent the development of an oxidized flavor cannot be ascribed to disappearance of the acid.

6. No single treatment or combination of treatments produced systematic changes in the pH consistently.

7. The two samples with the lowest initial Eh values were among the first to show an oxidized flavor after storage at 40° F. and -10° F.

8. Samples with a high initial Eh increased less while those with a low initial Eh increased more.

9. The two largest Eh increases accompanied the development of an oxidized flavor reported after 8 weeks storage at 40° F. were accompanied by a small increase and a slight decrease in Eh.

10. The two samples showing an oxidized flavor after 8 weeks storage at 40° F. but not after 3 weeks at the same temperature both decreased in Eh. The oxidized flavor did not appear until the initial rise in Eh had passed its maximum.

11. The 6 samples whose Eh increased most after storage for 5 months at -10° F. developed an oxidized flavor either then or after 6 months storage. The one sample which had an oxidized flavor criticism at 5 months but not at 6 months showed a moderate Eh increase at 5 months and an additional increase at 6 months.

12. After 6 weeks storage at 40° F. oxidized flavor appeared in butter churned from spontaneously oxidized milk to which ascorbic acid had been added. Tocopherol protected this detrimental effect of ascorbic acid but was not shown to improve the control. All samples churned from spontaneously oxidized milk and none of the samples from non-spontaneously oxidized milk had developed an oxidized flavor after 3 months storage at -10° F. regardless of treatments.

13. The changes in pH and Eh of butter churned from spontaneously and non-spontaneously oxidizable milk were not large and probably not significant in relation to the oxidized flavor.

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SOME STUDIES ON THE EFFECTIVENESS OF ASCORBIC ACID AND TOCOPHEROL IN RETARDING OXIDATIVE RANCIDITY IN BUTTER

by

FRANKLIN JOSEPH HEIM

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The deterioration of dairy products due to oxidative processes in the fat phase has been a main problem for research in dairying for many years. Carson and Thurston (1), Greenbank (3). Research in retarding or preventing this deterioration has taken several different directions, one of which is the use of antioxidants.

Previous work by Chilson (2), who studied the effectiveness of added ascorbic acid to butter in preventing oxidative rancidity, showed that the butter developed an oxidized flavor while there was sufficient ascorbic acid present to normally retard oxidation. A significant correlation (.51) was found by Krukovsky, Loosli, and Whiting (4) between the tocopherol content of milk fat and the ability of milk to resist the reaction, involving ascorbic acid oxidation, which produces oxidized flavors. The special physical structure of butter makes it impossible to draw any conclusions with regard to butter from experiments with milk, butterfat or vegetable oils.

This study was therefore undertaken to obtain additional information on the effectiveness of ascorbic acid and the possible synergistic activity of ascorbic acid and d,l-alpha-tocopherol in preventing oxidative rancidity in unsalted stored butter.

It was planned to study the development of an oxidized flavor in the fresh and stored butter by organoleptic tests and possibly find some correlation between the flavors occurrence and a change in the pH, Eh and ascorbic acid content of the butter

sera. Three commercial size churnings were made, aliquots of butter were taken from the churn and various amounts of ascorbic acid, tocopherol and copper were worked into the samples. Each aliquot was then split into four parts, two of which were stored at 40° F. and two at -10° F. for various periods up to six months. The tests included organoleptic tests to determine flavor deterioration, pH and Eh measurements, and ascorbic acid determinations.

When oxidized flavor appeared in the market milk two groups of cows were selected, by flavor tests, to produce milk for experimental hand churnings. These cows were classified as either producing spontaneously oxidized milk or non-spontaneously oxidized milk. The milk was separated, the cream churned and ascorbic acid alone, tocopherol alone, or both were added. The samples were then stored at 40° F. and -10° F. for various storage periods.

The data was then collected and an attempt made to correlate the various treatments with the oxidized flavor and also compare the flavor with pH, Eh and ascorbic acid content. A summary of the experimental results shows:

1. None of the samples whose change in score was equal to or less than the control developed an oxidized flavor. Addition of copper was the only treatment which produced a larger drop in flavor score without producing an oxidized flavor.

2. In three samples with the least drop in flavor score treatment with ascorbic acid and tocopherol apparently minimized other off flavors but not the oxidized flavor.

3. Ascorbic acid plus copper produced a larger flavor score drop than the control after six months storage at -10° F.

4. The final ascorbic acid content of samples with a small amount of added ascorbic acid was higher after eight weeks storage at 40° F. if tocopherol was present. With no added ascorbic acid or with a larger addition the final ascorbic acid values were lower when tocopherol was present.

5. Five out of six samples which had ascorbic acid added developed the oxidized flavor after eight weeks at 40° F. The final ascorbic acid concentrations were from 1.9 to 2.9 times the concentrations of ascorbic acid in the control. Thus the failure of ascorbic acid to prevent the development of an oxidized flavor cannot be ascribed to disappearance of the acid.

6. No single or combination of treatments produced systematic changes in the pH, consistently.

7. The two samples with the lowest initial Eh values were among the first to show an oxidized flavor after storage at 40° F. and -10° F.

8. Samples with a high initial Eh increased less while those with a low initial Eh increased more during storage.

9. The two largest increases in Eh accompanied the development of an oxidized flavor.

10. The two samples which showed an oxidized flavor after eight weeks storage at 40° F. but not after three weeks at the same temperature both decreased in Eh from three to eight weeks.

11. The six samples whose Eh increased most after storage for five months at -10° F. developed an oxidized flavor.

12. After six weeks storage at 40° F. an oxidized flavor appeared in the butter churned from spontaneously oxidized milk to which ascorbic acid had been added. Tocopherol, in this case, protected this detrimental effect of ascorbic acid but was not shown to improve the control. All samples churned from spontaneously oxidized milk and none of the samples from nonspontaneously oxidized milk had developed an oxidized flavor after three months storage at -10° F., regardless of treatment.

13. The changes in pH and Eh of butter from individual churnings were not large and not significant in relation to the oxidized flavor.

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