Addition of Clarase or pig mucosa to soybean meal slurries digested in water (pH 6.5) caused loss of added thiamine. No loss was observed by addition of wheat germ phosphatase, or by autolysis of the raw meal. The cause of losses by Clarase and pig mucosa was not apparent. Likewise, the good recovery with wheat germ phosphatase is not understood.

About 60% of the thiamine in soybeans is present in bound form presumed to be cocarboxylase. Raw meal contains a phosphatase with optimum activity at pH 6.0 which liberates free thiamine from cocarboxylase on autolysis. In the Johnson analysis, soybean meal is digested at pH 4.5 with Clarase to liberate thiamine from its phosphate form, and with papain presumably to liberate protein-bound thiamine. No additional thiamine was liberated by papain, trypsin, or pepsin; hence, their presence in the digestion is apparently unnecessary. The use of Clarase or some other phosphatase is advisable because cooking soybean meal for feeds inactivates the natural phosphatase; also the natural phosphatase is not very active at pH 4.5.

Destruction of meal thiamine with sulfite and with alkali, followed by addition of ferricyanide, failed to yield fluoresing materials in excess of those in the blank. Also, fluorescent materials obtained from the meal acted like thiochrome, in that they were entirely destroyed by ultraviolet irradiation. Hence, there was no evidence that fluorescence was not a measure of thiamine in the meal.

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QUALITY AND FLAVOR OF DAIRY PRODUCTS

Review of Biochemical Properties of Milk and the Lipide Deterioration in Milk and Milk Products as Influenced by Natural Varietal Factors

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THE FLAVORS OF MILK and milk products and their behavior in normal use seriously affect their consumption and may make them totally unfit for human food. The psychic effect of smell and taste cannot be lightly dismissed (*40*), and oxidized fats in the diet may lower the utilization of vitamin A and actually be injurious to health (*38, 44*). Consequently, it is very desirable to make milk products both palatable and relatively resistant to the causes of bad flavors and losses of nutritive value.

The prevailing tendency, however, is still to emphasize quantity production of feed and milk to reduce production costs, to promote consumption of milk by stressing its unique nutritive properties, and to lean largely on processing methods as a source of aid, rather than to improve production methods on the farm. Yet, fat-soluble vitamin content of milk and certain biochemical properties which control palatability and thus consumption are related not to quantity production of feed and milk, but to the type and quality of roughages and supplements fed, the physiological response of cows of a given breed to the feed consumed, and the handling of milk after withdrawal from the mammary gland.

Major Causes of Undesirable Flavors

Major causes of undesirable flavors in milk and milk products include: odorous principles or their metabolites which pass from the feed via the cow's blood to the milk, giving it a "feedy flavor"; splitting of fat by the milk enzyme lipase, products of which give milk a rancid odor and bitter taste and increase the acid degree of fat; and certain other chemical reactions, enhanced by specific substances or by exposure of milk and milk products to light, which result in metallic-to-fishy, oily, and chalky-to-soapy-tallowy (cardboard-like) flavors.

Lipolytic Rancidity in Raw Milk

Shortly before World War II, lipolytic rancidity caused by by-products of fat splitting was one of the greatest problems of the dairy industry. It was solved here at Cornell University by a careful study of the methods used for handling raw milk on the farm and in the milk plant.

The milk enzyme lipase was found to be activated by slight warming of cold milk and subsequent recooling; activity increased as the temperature was lowered

The purpose of this paper is to provide a perspective and better understanding of biochemical processes which control palatability and nutritive value of milk and milk products. Causes of loss in palatability of prime interest are: splitting of fat in raw milk by lipase, which gives rancid odor and bitter taste; and other chemical reactions, enhanced by specific substances or by exposure of milk to light, which result in metallic-to-fishy, oily, and cardboard-like flavors. The importance of each varies with the method of handling raw milk, the type of feed the cow eats and her physiological response to the feed consumed, the type of product held, and processing and storage conditions. Although we are still in the first stage of solving the problem, several methods are available for producing palatable and nutritious milk and milk products.

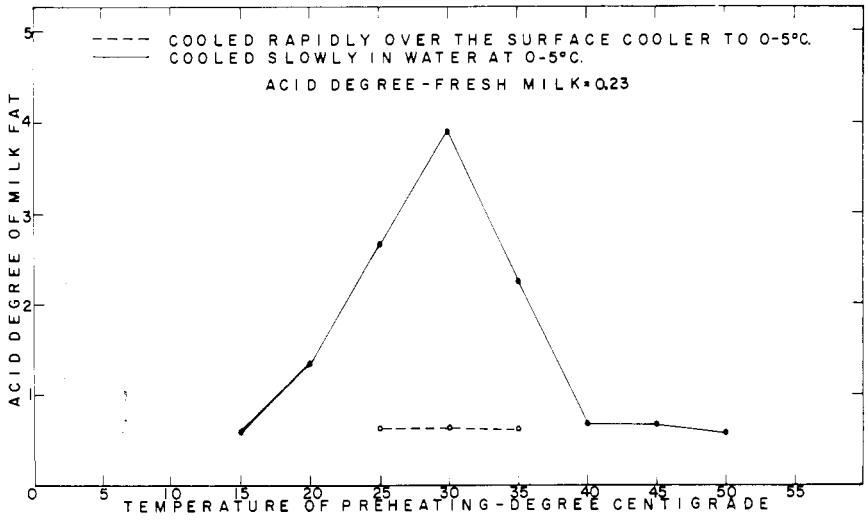

Figure 1. Activation of milk lipase by temperature changes

Holding time 24 hours

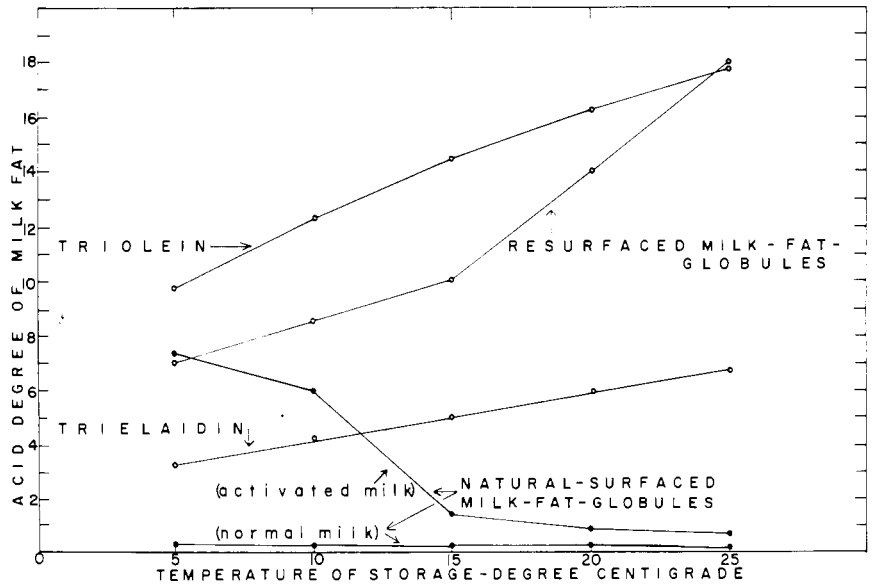

Figure 2. Effects of temperature of storage on rates of lipolysis in milk with natural-surfaced and resurfaced milk fat globules

Holding time 24 hours

(*28*, *29*). Maximum activation occurred when raw milk originally below 10° C. was warmed to 30° C. and then slowly recooled to below 10° C. (Figure 1). Milk so treated became unfit to drink, sometimes shortly after milking. This could be prevented by cooling both the fresh and the temperature-activated milk rapidly to below 10° C., and holding it there until pasteurization; this caused the enzyme to be reversibly inactivated.

Lipase can be inactivated irreversibly and completely by heating milk at 58° to 63° C. for 30 minutes in the presence of occluded oxygen (*35*), by rennet and acids at the isoelectric point of casein (*20*), and by preformed H_2O_2 added in amounts needed to oxidize all of the ascorbic acid in the milk to dehydroascorbic acid (*21*).

Splitting of milk fat occurred also when the raw milk was allowed to churn and the fat to resurface on shaking or foaming (*20*, *33*). The lipolysis of the milk-fat globules resurfaced by churning of cream and the re-emulsification of pure fat in raw skim milk, as well as of triolein and trielaidin substrates, proceeded faster, however, as the incubation temperature increased (Figure 2), showing a "positive temperature coefficient" (*34*), whereas the lipolysis of natural-surfaced milk-fat globules in temperature-activated milk accelerated as the temperature decreased, showing a "negative temperature coefficient." Triolein and trielaidin were used for comparative purposes. They are simple glycerides with well defined and widely different melting points, while the mixed glycerides form the bulk of milk fat, and their composition varies with feed, breed differences, and the response of individual cows of a specific breed to the feed.

Consequently, the observed variations in the susceptibility to lipolysis of natural-surfaced and resurfaced milk-fat globules, olein, and trielaidin (Figure 2) indicate that the "negative coefficient" for lipase action in raw milk is associated with the natural fat-globules-stabilizing membrane, and that the extent and magnitude of lipolysis are greatly influenced by the physicochemical properties of fat. The lipolysis of resurfaced milk-fat globules in raw milk can be avoided only by pasteurization.

Likewise, the deaeration of raw milk reactivates lipase, doubling its activity and lessening the destruction of enzyme by heat at 43° to 63° C. (Figure 3). In deaerated milk, dissolved copper exerts no destructive action on lipase, and it accelerates the destruction of lipase by heat in the presence of occluded oxygen only (*35*).

The deaeration of raw milk also extends the protective influence against

photochemical inactivation of milk lipase (*21*). In the presence of occluded oxygen the enzyme sensitivity to light varies appreciably, however, among samples of milk from individual cows (*21*).

The origin of bitter flavor in rancid milk was traced by us to the gravity cream layer of raw milk (*21*). Since the milk fat agglutinin concentrate (*22*) obtained by the author via reseparation of gravity cream at 37° to 40° C. had a tendency to develop bitter flavor upon standing at refrigeration temperatures, and the fat itself from rancid milk was often bitter, it was assumed that the by-products of fat splitting and degradation of migratory (*20, 22*) components of the fat-globules membrane are both responsible for bitter flavor. The bitter principles and milk enzyme lipase are very unstable and do not yield to the isolation techniques now in use.

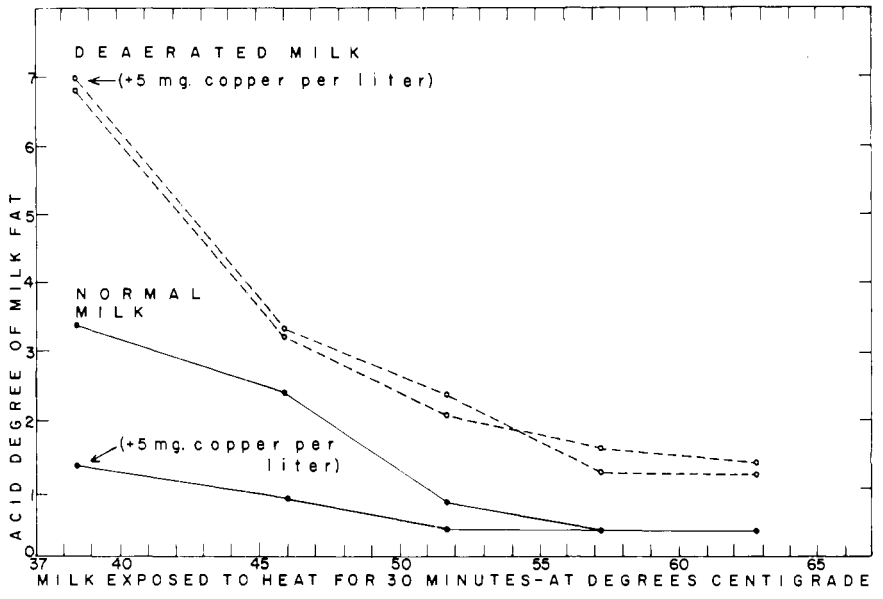

Figure 3. Effects of exposure to heat of copper-treated and untreated normal and deaerated milk on rates of lipolysis in activated milk held at 0° to 5° C. for 48 hours

Oxidative Deteriorations

Deterioration of Pasteurized Milk and Its Products. Another problem has been the stabilization of milk, cream, and butter against oxidative changes which affect palatability and nutritive values. The flavor defects related to such changes, often identified as oxidized flavors (*7*), actually consist of nonpersistent metallic-to-fishy flavors (*8, 12, 16, 36, 37*) associated with deterioration of unstable flavor-forming principles (phospholipides) oriented on the surface of the fat globules (*8, 42, 43*), and oily and chalky-to-soapy-tallowy (cardboard) flavors associated with deterioration of flavor-forming principles (*7*) localized in the skim milk phase of the whole milk (*5, 8, 16*). These flavors in fresh milk or buttermilk may supersede each other in the order given (*8, 12, 16, 36, 37*). The successive changes in the flavor characteristics of milk, which are catalyzed by copper and light, can be traced

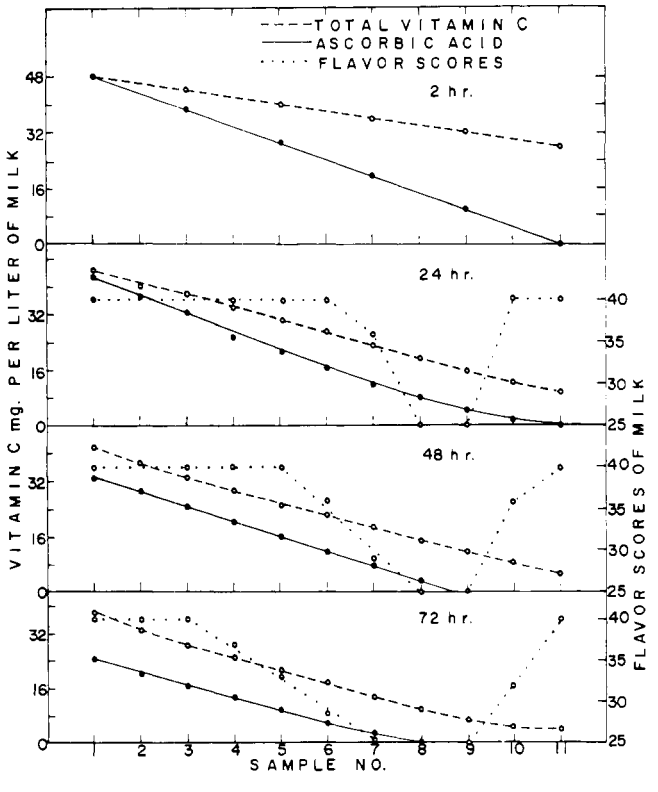

Figure 4. Ascorbic-dehydroascorbic acid equilibrium in milk after storage at 0° to 5° C. and promotion of harmful-to-flavor reactions

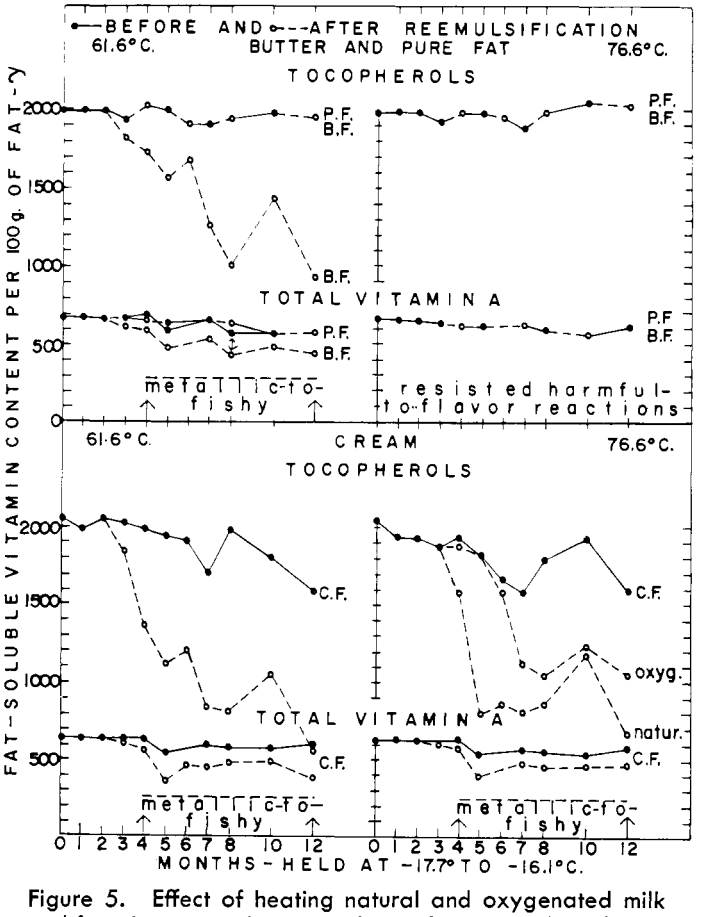

Figure 5. Effect of heating natural and oxygenated milk and freezing cream, butter, and pure fat on stability of vitamins A and E and susceptibility of fat from these products to harmful-to-flavor reactions as determined by re-emulsification

readily in buttermilk from oxidation-sensitive milk (Table I). The off-flavors are more intense in buttermilk than in milk, because the flavor-forming principles, with other migratory components of the fat-globules membrane (agglutinin and natural and heat-degraded flavoprotein), concentrate in the buttermilk during churning of cream (*20, 22*).

In fresh milk, fat is relatively stable and the oxidized flavors are not associated with its deterioration. However, the fat does deteriorate when milk products are used after prolonged storage, persistent metallic-to-fishy flavors develop, and in fat soluble vitamins are lost (Figure 5 and Table III). The likelihood of deterioration depends on the type of product held and upon production, processing, and storage conditions (*9–12, 27*). Fat also deteriorates when whole milk is permitted to oxidize with 5 to 10 p.p.m. of copper as catalyst (*8, 16*). In such a case, however, material for the development of metallic-to-fishy flavors is provided by the flavor-forming principles oriented on the surface of the fat globules, and by the fat itself. This is readily seen in the recent studies of Forss, Dunstone, and Stark (*4*) concerning the development of metallic-to-fishy flavors in washed cream oxidized with ascorbic acid and 5 p.p.m. of copper as catalysts.

Vitamin C and oxygen together (but not individually) play an important role in these harmful-to-flavor reactions, which are enhanced by light and copper, especially when the unstable fat is used in foods such as strawberry (*39*) and citrus fruit ice cream and reconstituted milk (*12, 27*).

The depletion of milk of the total vitamin C content by rapid oxidative methods postpones harmful-to-flavor reactions for a significant time (*9–11, 15, 18, 19, 25–27*). One method involves oxidation of all of the ascorbic acid in the milk to dehydroascorbic acid either by preformed H_2O_2 or photochemically by exposure to light, followed by pasteurization at 61.6° C. for 30 minutes. Another method is oxygenation of milk during pasteurization (*9, 10, 18, 27*). Dehydroascorbic acid is the unstable form of vitamin C and is readily destroyed by heat. However, the harmful-to-flavor reactions are promoted by quick-partial oxidation of ascorbic acid to dehydroascorbic acid photochemically or by preformed H_2O_2, and by addition of ascorbic acid and copper to milk entirely depleted of its total vitamin C content (*9–11, 25–27*).

The part which vitamin C plays in the harmful-to-flavor reactions is readily seen from Table I. These data were obtained by the author more recently, using oxidation-sensitive milk produced on legume–grass silage ration. They confirmed our earlier observations and show that in buttermilk depleted of its total vitamin C content by H_2O_2 and heat and then fortified with ascorbic acid and copper, the oxidized flavors supersede each other in the order: metallic-to-fishy, oily, and chalky-to-soapy-tallowy (cardboard-like).

Furthermore, the harmful-to-flavor reactions might be initiated more rapidly when a certain equilibrium between the two forms of vitamin C was established. To test this theory, a series of samples containing variable amounts of ascorbic and dehydroascorbic acids was prepared by diluting milk containing mostly ascorbic acid with identical milk in which all of the ascorbic acid had been oxidized to dehydroascorbic acid by preformed H_2O_2 (Figure 4). The harmful-to-flavor reactions were initiated more rapidly when the ratio of ascorbic to dehydroascorbic acids was approximately 1 to 1 or lower, as shown by the flavor scores of milk (40 = no criticism; 25 = completely unpalatable). In addition, it became evident that an unfavorable proportion of dehydroascorbic acid could not be accumulated if the rate of its oxida-

Table I. Effect of Vitamin C on Harmful-to-Flavor Reactions

Treatment of Milk	Milk Held at 0°–5° C., Days	Cu, Mg./L.	Natural Vitamin C: Ascorbic acid, mg./l.	Natural Vitamin C: Total vit. C, mg./l.	Natural Vitamin C: Flavor criticism	Added Vitamin C: Ascorbic acid, mg./l.	Added Vitamin C: Total vit. C, mg./l.	Added Vitamin C: Flavor criticism
Natural milk	0	...	17.1	17.6	−	...	...	...
	7	...	11.2	12.6	−	...	...	...
	2	0.1	3.3	8.0	+++	...	...	...
H_2O_2[a]	0–7	0.1	...	0.0	−	...	...	...
	0	0.1	...	...	...	20.0	...	−
	1	0.1	...	...	...	5.0	12.1	+++
Photochem.[b]	0–7	0.1	...	0.0	−	...	...	...
	0	0.1	...	...	...	20.0	...	−
	1	0.1	...	...	...	9.1	17.3	+++
Oxygenation[c]	0–20	0.1	...	0.0	fresh	...	...	...
	0	0.1	...	...	...	20.0	...	−
	1	0.1	...	...	...	1.0	13.1	+++
Buttermilk (H_2O_2-milk)	0–7	0.5	...	0.0	−	...	...	...
	0	0.5	...	...	...	20.0	...	−
	1–7	0.5	...	...	...	...	...	(+++)

[a] 0.03 ml. of 30% H_2O_2/l.
[b] Exposed to sunlight for 30 minutes, then heated at 61.6° C. for 30 minutes.
[c] Oxygenated during heating at 61.6° C. − No criticism. +++ Metallic. (+++) Metallic-to-fishy, then oily and chalky-to-soapy-tallowy (cardboard-like).

Table II. Effects of Heating Natural and Oxygenated Milk on Harmful-to-Flavor Reactions in Frozen Cream and Butter

Milk	°C.	Product Held	Months Held at −17.7° to −16.1° C. 0	1	2	3	4	5	6	7	8	10	12
			Palatability Ratings										
Natural	61.6	Cream	−	−	−	+	+	+	−	+	+	+	+++
		Butter	−	−	−	−	−	−	−	−	−	−	−
Natural	76.6	Cream	−	−	−	−	−	−	+	+++	+++	+++	+++
		Butter	−	−	−	−	−	−	−	−	−	−	−
Oxygenated	61.6 and 76.6	Cream	−	−	−	−	−	−	−	−	−	−	−
		Butter	−	−	−	−	−	−	−	−	−	−	−

− No criticism. + Slight loss in palatability. +++ Unpalatable.

Table III. Effects of Heating Natural and H_2O_2-Treated Milk and Freezing Cream, Butter, and Pure Fat at −17.7° to −16.1° C.

Natural and H_2O_2-Treated Milk Heated 30 Min. at °C.	Type	At End of 2 Years at −17.7° to −16.1° C. Original Product: Palatability	Original Product: Vitamins, μg./100 G. Fat: Total A	Original Product: Vitamins, μg./100 G. Fat: E	Fat Re-emulsified in Skim Milk (4%)[b]: Palatability	Fat Re-emulsified in Skim Milk (4%)[b]: Vitamins, μg./100 G. Fat: Total A	Fat Re-emulsified in Skim Milk (4%)[b]: Vitamins, μg./100 G. Fat: E
61.6 and 68.3[a]	Butter	−	726	2307	+++	386	878
71.1 and 76.6[a]	Butter	−	760	3008	−	723	2965
All temp.	Cream	(−)	669	2407	+++		
	Fat	−	741	3047	−	717	2736

[a] 0.03 ml. of 30% H_2O_2 per liter of milk. [b] 20 mg. ascorbic acid and 0.1 mg. copper per liter. − No criticism. +++ (Unpalatable), metallic-to-fishy. (−) Cream from H_2O_2-treated milk only.

tion to nonreducible substances surpassed that of ascorbic acid oxidation to dehydroascorbic acid. Consequently, the protective influence of ascorbic acid added in large but variable quantities to different foods, including milk, could be attributed to the exhaustion of occluded oxygen prior to the establishment of a favorable equilibrium between these two forms of vitamin C.

Of interest are the findings of Snyder (*41*) and Greenbank (*7*) that ascorbic acid oxidation in synthetic media or milk is accompanied by a gradual increase in E_h up to the point where all of the ascorbic is oxidized to dehydroascorbic acid, whereas addition of ascorbic acid causes the potential to drop immediately by an amount which depends on the concentration of ascorbic acid added. This suggests that E_h might reflect these changes in ascorbic-dehydroascorbic acids content of milk (*7, 31*).

There is good reason to believe that the oxidation of ascorbic acid in the milk to dehydroascorbic acid by preformed H_2O_2 is catalyzed by peroxidase. The rate of ascorbic acid oxidation by H_2O_2 was found to be progressively retarded if the reagent was added to milk in excess of the amount needed to oxidize the vitamin rapidly and completely; while the subsequent heating of so-treated milk at 61.1° C. for 30 minutes inhibited this reaction, as tested by the addition of both reagents to milk after heat treatment (*11*). These results show that the reaction is catalyzed by an enzyme, which in turn is inactivated by H_2O_2 and heat. Likewise, the H_2O_2 was not utilized to oxidize ascorbic acid in the milk heated to 76.6° C., the temperature at which, according to Zilva (*46*), the inactivation of peroxidase should be rapid and complete. The reaction was induced again, however, by the addition of horseradish peroxidase to nonreactive milk (*15*).

Deterioration of Fat in Cream and Butter. In our study of the stability of vitamin A and its precursor, carotene, in milk fat (*23, 24*), it was pointed out that the re-emulsification of fat in pasteurized skim milk was very useful not only in recognizing the flavor defects of fat but also in detecting changes in its resistance to harmful-to-flavor reactions, which are enhanced by light and copper in the presence of ascorbic acid, whether these changes are brought about during storage or by photochemical destruction of vitamin A or any other factor.

By means of this test we were able to determine the behavior of frozen cream and butter during storage as influenced by the processing and storage conditions. While the creams depleted of the total vitamin C content, indirectly, through oxygenation of milk at 61.6° and 76.6° C. for 30 minutes, were found by judges to be perfect in flavor during 12 months' storage at −17.7° to −16.1° C. (Table II), the fat in both natural and oxygenated creams became unstable and underwent deterioration in the re-emulsification test at the end of 4 and 5 months, respectively (*9, 10*), as shown by development of a persistent metallic-to-fishy flavor and losses in fat-soluble vitamins (Figure 5). Depletion of the total vitamin C content by preformed H_2O_2 and pasteurization at 61.6°, 68.3°, 71.1°, and 76.6° C. for 30 minutes (*9, 10, 11, 27*) prevented oxidized flavors in cream even after 2 years (Table III). After that time these creams developed a slight nutty flavor, at the surface.

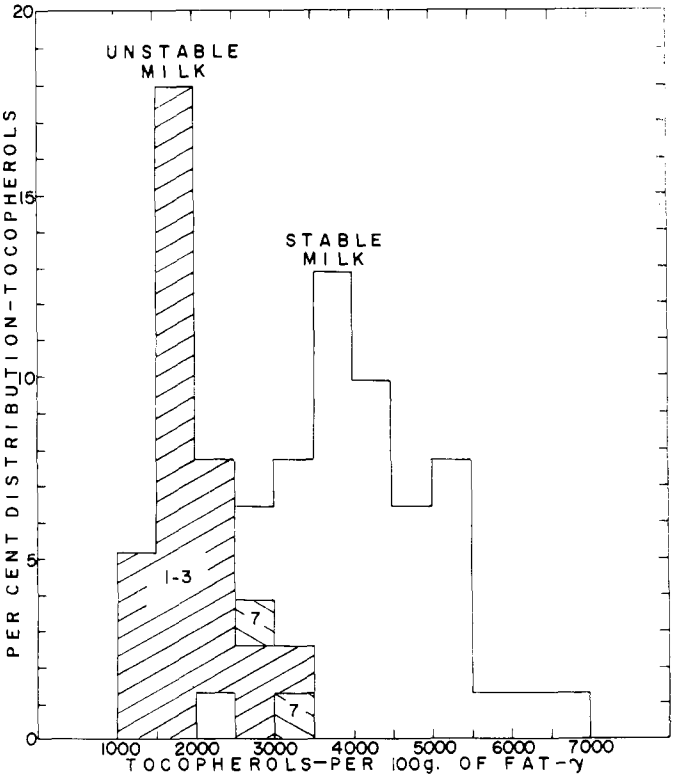

Figure 6. Distribution of tocopherols in 77 samples of milk as affected by tocopherol and cod liver oil supplements and ability of milk to resist harmful-to-flavor reactions during 7 days' storage at 0° to 5° C.

1-7. Day milk became unpalatable

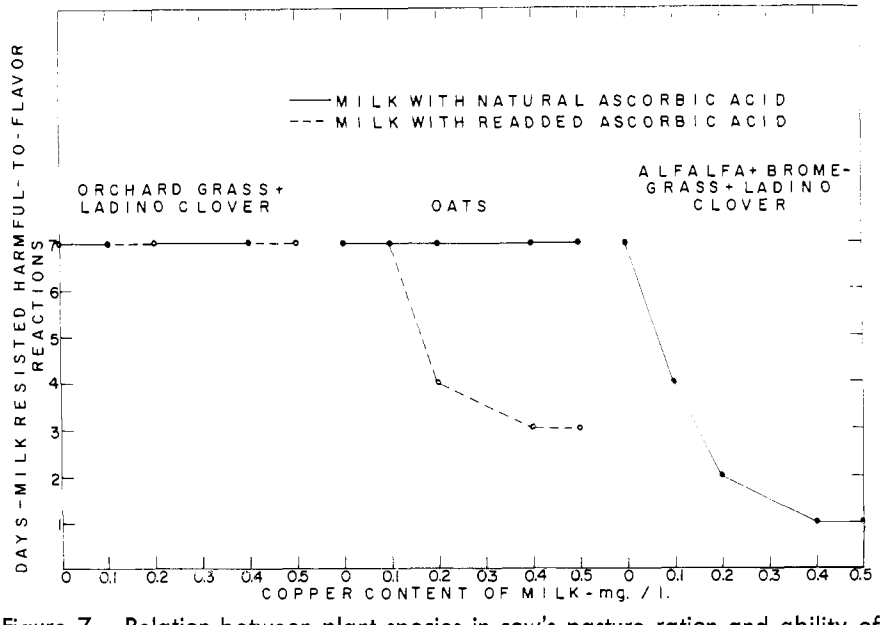

Figure 7. Relation between plant species in cow's pasture ration and ability of milk to resist harmful-to-flavor reactions during 7 days' storage at 0° to 5° C.

Table IV. Influence of Roughages on Ability of Milk to Resist Harmful-to-Flavor Reactions in Presence of 0.1 Mg. per Liter Added Copper as a Catalyst

Distribution of Milk Samples, %	Type of Roughages Fed				
		Mixed Legume-Grass Stand[a]			
	Prefeeding control	Early silage	Late silage	Early barn-cured hay	Late field-cured hay
	Day Milk Became Unpalatable during 10 Days' Storage at 0–5° C.				
50	2	2	—	2	2
8	2	—	—	2	—
17	—	2	—	—	—
25	—	—	—	—	—
	Av. Tocopherol Content per 100 G. of Milk Fat				
	3090 ± 263	2614 ± 323	2120 ± 554	1837 ± 501	

[a] Legume 55–60%, grass 25–30% (predominantly medium red clover and timothy with some alfalfa, alsike clover, red top, bluegrass, and orchard grass), yellow rocket weed approx. 16%. — Stable milk.

Irrespective of the temperature of pasteurization, the milk fat held in the form of cream loses its ability to resist harmful-to-flavor reactions in the re-emulsification test at much faster rate than the fat in butter; and only the fat in butter churned from cream pasteurized at 76.6° C. (Figure 5) and 71.1° or 76.6° C. (Table III) and the pure milk fat were nonsusceptible to deterioration and thus remained stable after 1 and 2 years' storage, respectively, at −17.7° to −16.1° C.

The above described behavior of cream and butter has also indicated that their organoleptic properties during storage may bear no relationship to the stability of fat as determined by the re-emulsification test.

Influence of Roughages on Biochemical Properties of Milk

The kind of feed the cow eats, the breed, and the physiological response of the individual cow within a breed to the feed consumed all have significant effects upon vitamin A, carotene (*2*, *10*, *31*, *32*, *36*), and tocopherol (*10*, *30*, *31*, *32*, *36*) levels of the milk fat, palatability, and biochemical properties of milk related to its stability (*10*, *12*, *30*, *31*, *32*, *36*). These factors were found to have statistically significant effects on tocopherol, vitamin A, carotenoid, iodine, and thiocyanogen values and the refractive index of milk fat (*13*, *36*).

Moreover, the level of milk tocopherols was shown to be positively correlated with milk carotenoids (*32*) as affected by both pasture and barn feeding (+0.68 and +0.63), and the ability of milk to resist harmful-to-flavor reaction (*31*) as affected by tocopherol and cod liver supplements (+0.51) (Figure 6); a negative correlation was also indicated between carotenoid level and the iodine value of fat, when different rations were fed in a consecutive order to a group of dairy cows of four breeds (*13*).

However, the resistance of milk to the development of oxidized flavors was not directly correlated with tocopherol level alone (+0.51). Milk produced on late-cut silage retained its ability to resist oxidized flavors when stored in the presence of copper for 10 days (Table IV). In contrast, the resistance of milk to oxidized flavors was appreciably reduced when early-cut silage, barn-cured hay, and late-cut field-cured hay were fed. Fifty per cent of these became unpalatable after 2 days of storage. The early-cut silage produced milk with an average of 3090 μg. of tocopherol per 100 grams of milk fat; the late-cut silage, which produced more stable milk, contained an average of 2614 μg. of tocopherol. Thus roughages appear to contain variable amounts of substances in addition to tocopherols that extend their protective influences to milk and some of the cows continued to secrete these substances long after the causative ration had been discontinued (*36*).

It cannot be stated at present whether or not the seasonal trends for the plant species were primarily responsible for the behavior of milk produced on late-cut silage ration. The probability of this specificity was lessened by observations by the author that mold mycelium grew rapidly in early-cut silage samples to give it a grayish appearance, but not in the late-cut silage, when both were brought in for chemical analysis. The late-cut silage contained relatively large amounts of pods with seeds from the yellow rocket weed, which could have been a contributing factor.

In another experiment (*17*) Holstein cows on predominantly orchard grass with some ladino clover, green oats, and alfalfa–brome grass–ladino clover pastures produced an average of 3044, 3449, and 3411 μg. of tocopherols per 100 grams of milk fat, and of 41.07, 36.30, and 39.96 iodine numbers, respectively. However, the milk had different susceptibilities to oxidized flavors (Figure 7), especially in the presence of 0.1, 0.2, 0.4, and 0.5 mg. of copper per liter and of 20-mg. portions of ascorbic acid re-added to milk at the point of its depletion. Orchard-grass milk was exceptionally stable. It resisted oxidized flavors through the seventh day of trial in the presence of 0.5 mg. of copper and 3 portions of added ascorbic acid. Milk produced on alfalfa–brome grass–ladino clover pasture was the least stable. It developed oxidized flavors at the end of 4, 2, and 1 days with increase in copper content alone from 0.1 to 0.4 mg., respectively. Feeding of oats pasture resulted in the stabilization of milk against harmful-to-flavor reactions induced by copper, and in their deferment for 4 and 3 days upon re-addition of ascorbic acid to milk containing 0.2 to 0.4 mg. of copper.

Thus evidence was obtained once again that the onset of the harmful-to-flavor reactions in fresh milk might be postponed or prevented, depending on the presence of as yet unknown substances, secreted into the milk under certain feeding conditions, which in addition to tocopherols extend their protective influence to milk; the copper content of milk; and the availability of ascorbic acid; and that in the presence of ascorbic acid and copper these protective substances and the flavor-forming principles are oxidized preferentially. These conclusions could also be derived from earlier observations of Whitnah, Martin, and Beck (*45*), that although all samples of milk which developed oxidized flavors were below the breed average in color of fat, some samples low in color did not develop oxidized flavors; from the organoleptic behavior of milk fat fractions (*14*); and from Dahle's (*3*) observations that the onset of the harmful-to-flavor reactions was not delayed by the addition of carotene to butter.

Milk of poor keeping quality resulted during the ladino clover (70%) plus orchard grass (20%) feeding (both pasture and hay), whereas the transfer of cows from predominantly ladino clover to bird's-foot trefoil (47%) plus bluegrass (52%) pasture resulted in the stabilization of milk against harmful-to-flavor reactions (*30*). A gradual increase was observed by the author in the tocopherol content of milk fat from an average of approximately 2938 μg. per 100 grams of fat at the end of ladino pasture to a plateau level of 4350 μg. during the third and fourth weeks of bird's-foot–grass pasture. The transfer of the same cows from the pasture to hay feeding in the barn manifested itself by a relatively uniform decrease in the tocopherol content of the fat to an average of 2138 μg. but a variable susceptibility of milk to the development of oxidized flavors, depending on the kind and quality of the hay fed. Ladino clover hay produced unstable milk. In contrast, mixed legume-grass hay such as alfalfa (32%) plus blade-type grasses (59%), bird's-foot trefoil plus bluegrass hay, and timothy hay produced a relatively stable milk.

In general, pasture, silage, and hay rations made up of predominantly blade-type grasses, or good quality corn silage, appear to produce relatively stable milk, whereas early medium red clover, ladino clover, and soybean silage in the cow's ration render milk unstable. The influence of roughages on the ability of milk to resist harmful-to-flavor reactions was recognized by earlier workers in the field, such as Anderson, Wilson, and Hardenbergh (*1*), Whitnah *et al.* (*45*), and Garrett, Hartman, and Arnold (*6*), whose work indicated that a relatively stable milk was produced when machine-cured alfalfa, dehydrated young oats plants, and molasses grass silage, respectively, were included in the ration.

Palatability Ratings of Frozen Cream and Butter

Of particular interest was a recent finding that, with passage of time, frozen cream and butter from Jersey cows had higher ratings than those from Ayrshires when the cows were all fed the same ration, such as second-cut good quality alfalfa hay plus corn silage (Figure 8), whereas Brown Swiss and Holstein-Friesian products were intermediate. As an average, Ayrshire milk fat was lower in carotenoids (524 μg. per 100 grams of fat) and higher in iodine value (33.0) than that of any other breed; Jersey fat was highest in carotenoids (800 μg.) and lowest in iodine value (28.50); Brown Swiss and Holstein fats were again intermediate. Their total vitamin A content, however, remained at approximately the same level. This relationship between the milk fat constants held also when different rations were fed in a consecutive order to a group of dairy cows of four breeds (*12*).

Likewise, the cream and butter from milk produced by a single breed of cows on alfalfa hay plus corn silage ration had appreciably higher ratings during storage than those produced on legume-grass pasture made up of alfalfa, ladino and red clovers, brome grass, and bluegrass, whereas legume-grass hay and bird's-foot trefoil hay products were intermediate in that order (Figure 9).

In this study, it was considered desirable to pass the oxygen through the cream during its pasteurization at 71–73° C. in order to reduce the effects of different rations on initial palatability of cream and butter, and to minimize the chance of presence of other than feed and breed conditions. Under certain feeding conditions the by-products of incomplete oxidation of odorous principles absorbed from the roughage in the intestinal tract or their metabolites may pass via blood into the milk and thus impart the "feedy flavors" to the product. In this case, passing oxygen through the milk (*18*) or cream significantly improves the initial palatability of milk products; fresh cream and butter so treated were invariably found by judges to be perfect in flavor.

The palatability ratings of the frozen cream and butter were evaluated on the basis of per cent distribution of samples which at the end of each storage period at −24° to −27° C. were considered by judges to be perfect in flavor. However, no cream or butter developed oxidized flavors during the trial. These products were tested with typical consumers, including students from the College of Home Economics, Dairy and Food Science, and some children. Although

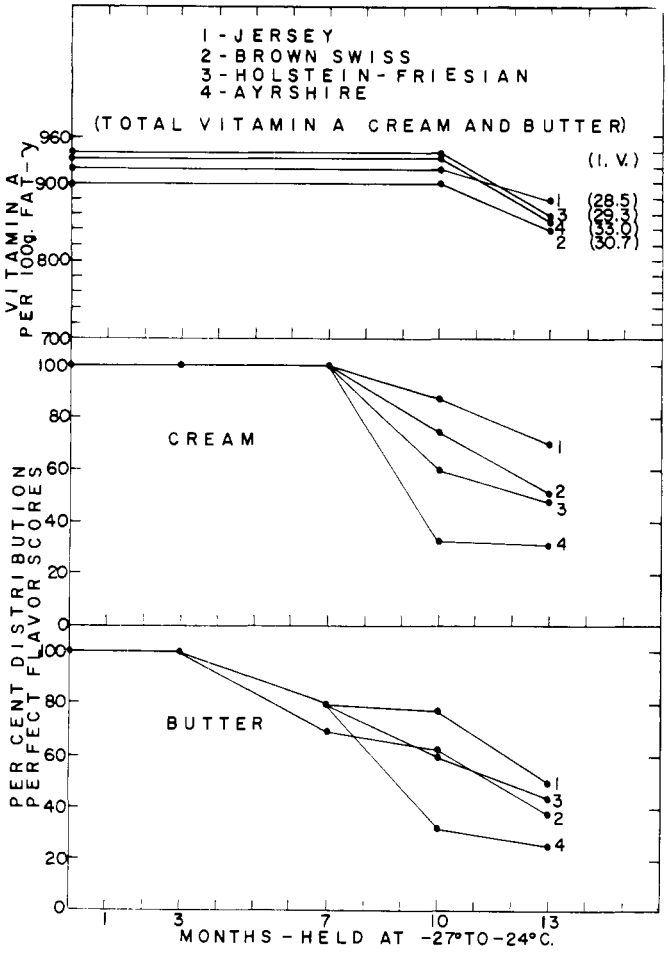

Figure 8. Influence of breed and heating of oxygenated cream at 71–3° C. on total vitamin A content of fat and palatability ratings of cream and butter during 13 months' storage

All cows fed same ration

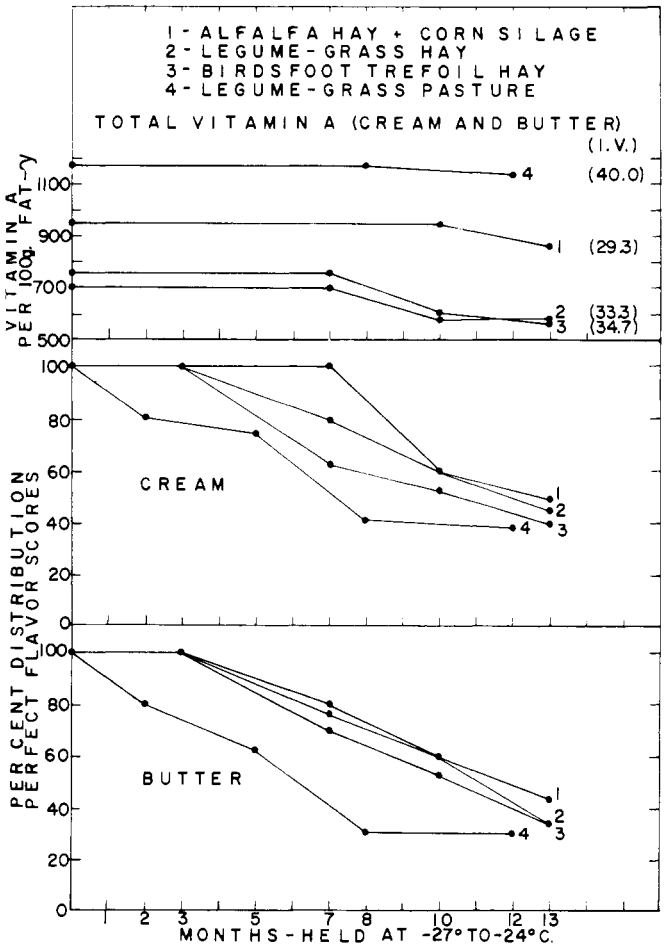

Figure 9. Influence of roughages fed to Holstein-Friesian cows and heating of oxygenated cream at 71–3° C. on total vitamin A content of fat and palatability ratings of cream and butter during 13 months' storage

the tests were conducted under normal conditions of use, all participants (approximately 30 individuals) made their judgments under the same environmental conditions. During the tests all participants were served tea (tea aids in the detection of flavor defects, which might well be missed otherwise, and alleviates the effects of preceding cream or butter on the participant's response to the next samples when a series is involved in the test).

Temperature of Storage and Stability of Fat

Although loss in the palatability of cream and butter held at −24° to −27° C. was followed by loss in total vitamin A contents (Figures 8 and 9), the re-emulsification of fat from the corresponding samples in the skim milk resulted in neither additional loss in vitamin A nor development of metallic-to-fishy flavors. In contrast, when cream and butter were pasteurized at 71.1° and 76.6° (Table III) and 76.6° C. (Figure 5), and t n h ld at −16.1° o −17.7° C., only the fat from butter and the pure fat were nonsusceptible to deterioration in the re-emulsification test at the end of 2 and 1 years' storage, respectively. The above behavior of cream and butter during storage indicates, therefore, that in addition to the heat treatment of cream and the type of product held, the stability of fat in the frozen products is also affected by the temperature of storage and that the organoleptic properties of frozen cream and butter may not necessarily bear a relation to the stability of fat subsequently used in the preparation of food.

Summary

Causes of loss in palatability of greatest interest are: splitting of fat in raw milk by lipase, which gives milk rancid odor and bitter taste; and other chemical reactions, enhanced by specific substances or by exposure of milk to light, which result in oxidized flavors. The importance varies with the method for handling raw milk, the type of feed and physiological response to feed, the type of product held, and processing and storage conditions.

Lipolytic rancidity is readily prevented if fresh milk is not allowed to churn and the fat to resurface by shaking or vacuum-foaming, or if raw milk is cooled rapidly to below 10° C. and held there until pasteurization. The enzyme is activated by slight warming of raw milk and recooling; the activity increases as the temperature is lowered, showing a negative temperature coefficient. Maximum activation occurs when milk originally below 10° C. is warmed to 30° C. and then slowly recooled to below 10° C. The rate of lipolysis of resurfaced fat globules increases with temperature, showing a positive temperature coefficient, and lipolysis can be avoided only by pasteurization. Deaeration of raw milk also reactivates lipase, doubling its activity and lessening the destruction of enzyme by heat.

Another problem has been the stabilization of milk, cream, and butter against reactions that produce nonpersistent metallic-to-fishy and oily flavors, which in fresh milk or buttermilk are associated with deterioration of flavor-forming principles oriented on the surface of the fat globules; and cardboard-like flavors which develop in the watery phase of the milk in the presence of variable amounts of copper.

In fresh milk the fat is relatively stable, but it may deteriorate after prolonged storage; persistent metallic-to-fishy flavors may develop and fat-soluble vitamins be lost. Deterioration depends on the type of product held, and on production, processing, and storage methods. In general, the fat in frozen cream loses its stability much sooner than the fat in frozen butter, and only the fat in butter churned from cream pasteurized at 71.1° and 76.6° C. and the pure fat remain stable for a significant time at subzero temperatures.

Vitamin C and oxygen play an important part in these deteriorative processes, which are enhanced by light and copper, especially when unstable fat is used in strawberry and citrus fruit ice cream or reconstituted milk. Rapid oxidative methods for depleting milk or cream of total vitamin C content (H_2O_2 or oxygen and heat) postpone harmful-to-flavor reactions for a significant time; oxygen also deodorized feedey flavors associated with odorous principles which pass occasionally from the feed via cow's blood to the milk. Quick partial oxidation of ascorbic acid to dehydroascorbic acid, either photochemically or by preformed H_2O_2, promotes these reactions. The organoleptic properties of frozen cream and butter therefore may bear no relation to the stability of fat subsequently used in foods.

The resistance of milk to harmful-to-flavor reactions depends in part on the tocopherol level, which in turn is greatly influenced by the roughages and supplements fed to the cows. Roughages also contain variable amounts of protective substances, some of which were secreted long after the causative ration had been discontinued. These substances are preferentially oxidized in the presence of ascorbic acid and copper. Thus the resistance of milk to harmful-to-flavor reactions that produce the oxidized flavors might be influenced by comparatively large or small concentrations of tocopherols and other protective substances.

In general, predominantly blade-type plants, such as orchard grass, oats, timothy, or good quality corn silage, produce relatively stable milk; early medium red clover silage, barn-cured hay, late field-cured hay, young alfalfa pasture, ladino clover, and soybean feeds render milk unstable.

The breed of the cows when all are fed the same ration and the type of ration fed to the same breed of cows influence the palatability of frozen cream and butter. Frozen cream and butter from Jersey cows had higher ratings than those from Ayrshires; Brown Swiss and Holstein products were intermediate. Ayrshire fat was lowest in carotenoid and highest in iodine value; Jersey fat was highest in carotenoid and lowest in iodine value; Brown Swiss and Holstein fats were intermediate. The cream and butter produced by a single breed of cows on alfalfa hay plus corn silage ration had appreciably higher ratings during storage than those produced on alfalfa, red and ladino clovers, bluegrass, and brome grass; legume-grass hay and bird's-foot trefoil hay were intermediate.

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FEED ADDITIVES

Microbiological Production of Carotenoids. Stabilization of β-Carotene in Dried Fermentation Solids

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Storage tests were conducted on stabilization of carotene produced intracellularly by mated cultures of the mold, *Blakeslea trispora*. Addition of 0.25% of Santoquin either to the medium during fermentation or to the dried product effectively stabilized carotene. Replacement of white grease with vegetable oil in the fermentation medium resulted in a more stable product in nonprotected solids. Effective stabilization was also achieved by suspending dried mycelium in vegetable oil, by storage under inert atmospheres, or in high vacuum. Addition of a chelating agent or incorporation of dried mycelium in gelatin or casein did not increase stability.

Carotenoid pigments in natural products used in animal and poultry feeds as a source of vitamin A and xanthophyll need to be stabilized. Current methods and materials, used with only limited success are: refrigeration, storage under inert atmospheres, control of moisture, and addition of antioxidants and animal fats or vegetable oils (*11*). The intracellular β-carotene of microorganisms intended for use as provitamin A in animal feeds, like β-carotene from other sources, must be protected against oxidation when exposed to air. No published information was found on stabilization of carotenoid pigments in microorganisms. The present investigation was concerned with the stabilization of β-carotene produced intracellularly by mating of appropriate heterothallic strains of a member of the order Mucorales, *Blakeslea trispora* (*2–4*).

Materials and Methods

Two strains of *Blakeslea trispora*, NRRL 2456 (+ mating type) and NRRL 2457 (− mating type) were utilized for all experiments. Inocula were produced in 500-ml. Erlenmeyer flasks containing 150 ml. of the following medium: acid-hydrolyzed corn, 2.3%; acid-hydrolyzed soybean meal, 4.7%; thiamine hydrochloride, 1.0 mg. per liter; sodium hydroxide to pH 6.2. The flasks were sterilized for 30 minutes at 121° C., inoculated with pieces of agar containing mycelium from 5- to 6-day-old potato-dextrose agar slants of each type, and then incubated for 2 days on a rotary shaker operating at 200 r.p.m. Two flasks of culture, one of each mating type, usually containing well dispersed growth, were combined as inocula for experimental media. A 10-ml. aliquot was used to inoculate each 100 ml. of fermentation medium. This medium had the same composition as that used for production of the inoculum, but in addition contained 0.12% of nonionic detergent (Triton X-100), 4% of cottonseed oil, and 0.1% of β-ionone; the latter compound was added 2 days after initiation of fermentation. After 4 days of additional incubation on a rotary shaker at 28° C., the mycelium was steamed for 10 minutes to destroy oxidative enzymes and then was recovered by filtration. Solids were dried in a vacuum oven at 50° to 55° C., ground in a Wiley mill equipped with a 20-mesh screen, and stored in 20-gram aliquots in 125-ml. Erlenmeyer flasks loosely stoppered with cotton.

Each antioxidant was dissolved in 3 ml. of diethyl ether and then poured over the ground solids. The antioxidant was distributed by trituration with mortar and pestle, while the ether was driven off. Only antioxidants of potential use in feeds were tested.

β-Carotene was determined by previously described methods (*2*).

Results

In a series of experiments eight antioxidants were evaluated for their ability to stabilize carotenoid pigments contained in the crude, dried fermentation solids. Data from storage tests at 28° C. following addition of 0.25% (w./w.) of a variety of antioxidants are shown in Table I. At a concentration of 0.25%,