Stability of Carotene at Elevated and Room Temperatures

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FOR several years an elevated temperature test (75° C.) has been used in this laboratory to facilitate the study of antioxidants as stabilizers for carotene in oil solutions. Only through the use of accelerated tests can rapid progress in evaluation of antioxidants be made. It is important, however, that the test used be a reliable index of the results to be expected from normal temperature storage over a longer period of time. At the time this work was begun, little information was available on the correlation between carotene stability at elevated and normal storage temperatures. Since then several papers have compared the efficiency of antioxidants for carotene at normal and at elevated temperatures, but only a few antioxidants have been thus compared and the results have not always been consistent.

Morgal *et al.* (11), using accelerated tests at 45° and 60° C., found lecithin to be effective for carotene. This result was confirmed by room-temperature storage tests although the relative order of stability of some of the samples varied with temperature of storage. Wall and Kelley (12) found that the addition of d-iso-ascorbyl palmitate and soybean lecithin to oil solutions of carotene did not increase the stability of carotene at 5°, 24°, and 37.5° C. whereas studies made by the Swift Stability method (7) at 100° C. indicated that these additives greatly increased carotene stability. Hove and Hove (6) showed that, under the conditions of their test, the relative antioxidant activities for carotene of gossypol, dianilinogossypol, and the three known naturally occurring tocopherols differed markedly with temperatures between 4° C. and 98 ° C.

The following communication reports comparative data on efficiency of antioxidants obtained with an elevated temperature test and with comparable tests at lower storage temperatures. The relationship between stability of carotene and temperature was studied primarily under a specific set of conditions, namely those of the accelerated test. In general, the accelerated test made possible a rapid selection of the better antioxidants and permitted a conservative estimate of increase in carotene stability to be expected at lower temperatures for most of the antioxidants studied. Certain antioxidants that appeared to be effective at the elevated temperature were, however, ineffective at the more common storage temperatures.

Reproducible results can be obtained with the accelerated test only if the numerous variables are maintained constant. Thus stability varies with concentration of the carotene (2, 12), its purity (4, 11), concentration of the antioxidant (naturally present or added) (2, 9, 11, 13), presence or absence of light (8, 9), and surface-volume relationship of the oil sample.

Furthermore, the composition of the oil used as carrier for the carotene and antioxidants will influence the stability of the carotene and the relative efficiency of the added antioxidants. In our experience oil used at different times, although stored at —30° C., oxidized sufficiently during storage to cause some decrease in stability. Hove and Hove (5) also found that the effect of age of a stock solution of oil was to decrease the stability of carotene subsequently tested in the oil even though the oil was stored at 10° C. By retesting several previously evaluated antioxidants with each series of tests, one can observe this change in oil. A better evaluation of antioxidants could be made if a standard reproducible fat substrate were available.

In order to assure accurate carotene analyses the colored oxidation products of carotene which develop during the storage period of the test were removed prior to the carotene determination (1). To eliminate another source of variation only new vials were used in all tests.

Methods

The details of the accelerated test have been published previously (13). Briefly, it consists of a determination of the time required for breakdown of 20% of the carotene in an oil solution (1.2 mg. crystalline carotene per g. of oil), stored as a thin layer at 75° C. under specified conditions, as measured by a chromatographic-colorimetric method.

In order to establish a relationship between accelerated and more normal conditions antioxidants that showed promise at the elevated temperature were tested at lower temperatures in portions of the same carotene-in-oil solutions. For 40° and 75° C. storage constant-temperature ovens were used. A large room, maintained at 25° C. and a relative humidity of 55-60% was used for the lower temperature storage. Sample vials were loosely stoppered with cotton plugs to exclude foreign materials. Wire racks each holding 40 vials were used to hold the storage samples. The samples stored in the 25° C. room were placed in cardboard boxes covered with wrapping paper to exclude light. In a few cases the samples were prepared and stored somewhat differently; the procedures used in these cases will be described later.

As in previous papers of this series (3, 13), symbols are used to represent the antioxidants and their combinations. The following symbols represent the various antioxidants tested:

A—l-ascorbyl palmitate (6-palmitoyl-L-ascorbic acid)
As—ascorbic acid
C—citric acid
H—hydroquinone
P—phospholipid (cottonseed)
N—*nordihydroguaiaretic acid*
T—alpha-tocopherol
V—Viobin

Example: TPH represents alpha-tocopherol, phospholipid, and hydroquinone used in combination. (Each antioxidant was added at the rate of 0.05% of the weight of the oil.) The constants for the oils used have been previously given (3). Coconut oil

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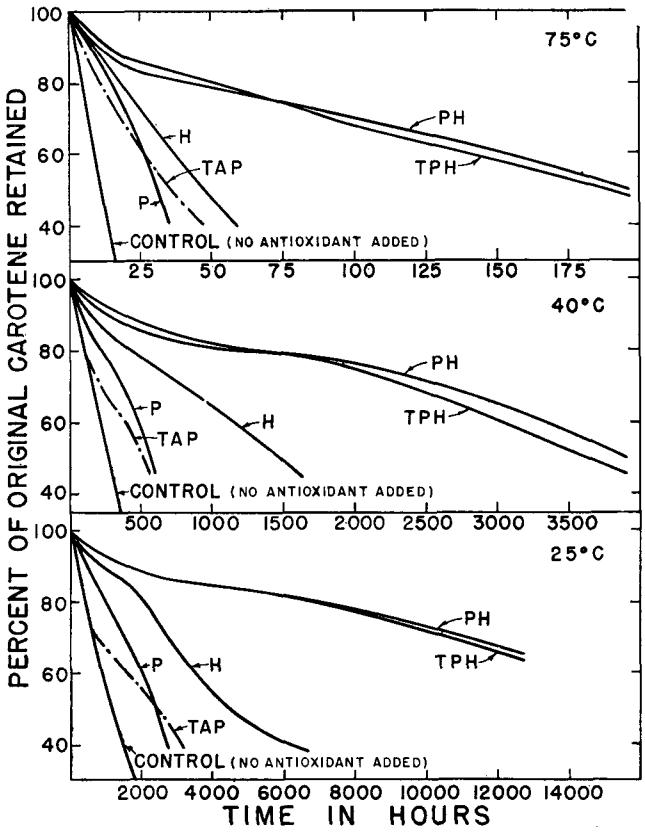

FIG. 1. Effect of storage temperatures and added antioxidants on the stability of carotene in refined cottonseed oil solution.

was a commercially refined oil. The lard was a prime steam-rendered lard supplied by Swift and Company. These were given no special treatment prior to use.

Carotene in Refined Cottonseed Oil

Figure 1 presents the curves for breakdown of carotene in refined cottonseed oil (Wesson) with and without added antioxidants, at 25°, 40°, and 75° C. In these storage tests there was no "induction period" as commonly reported in oil stability studies. Rather, in all cases studied, loss of carotene started at the beginning of the storage period and proceeded at various rates, depending on the temperature and on the presence and nature of added antioxidants.

As in the previous reports (3, 13), the time required for 20% loss of carotene is chosen as the reference point for comparing the stability of the various samples. For convenience in discussion, the time for 20% loss is called the STABILITY VALUE$_{20}$. In order to have a numerical measure of the relative effectiveness of the individual antioxidants and their combinations in the various oils, the ANTIOXIDANT INDEX is used. The index is obtained by dividing the time in hours for 20% loss of carotene in the treated sample (with added antioxidant) by the time for 20% loss in control.

Table I presents the STABILITY VALUES$_{20}$ and the ANTIOXIDANT INDICES for the antioxidants shown in Figure 1 at the three temperatures studied. The stability values depend on the point of reference chosen for comparison. In order to determine how the STABILITY VALUES$_{20}$ would compare with stability values obtained at the 50% breakdown point (STABILITY VALUE$_{50}$), analogous data at this point were obtained and are also presented in Table I.

Although the ANTIOXIDANT INDICES as determined from the STABILITY VALUES$_{50}$ showed good correlation with temperature, it has been previously pointed out (6, 13) that a comparison based on the time for 50% loss of carotene could be misleading in predicting effectiveness of an antioxidant during the initial period of storage, since the slopes of the carotene-breakdown curves are different with the various antioxidants. For instance, for the antioxidant combination TAP, predictions based on the STABILITY VALUE$_{50}$ determined either at 25° or 75° C. would indicate this combination to be more effective than is actually the case as measured by the STABILITY VALUE$_{20}$ at 25° C. Also, under practical storage conditions the primary interest is in the length of time that a solution may be stored with little or no loss of carotene since it would not be economical to store a solution until 50% of its carotene has been destroyed. For these reasons we prefer to base our comparative measurements on the STABILITY VALUES$_{20}$.

The rate of breakdown of carotene increases with increasing temperature. By plotting the logarithms of the STABILITY VALUES$_{20}$ of the control samples (oil with carotene but no antioxidant added) against temperatures, a straight-line relationship was obtained (Fig. 2). Corresponding curves for samples to which

TABLE I.

Relative Effectiveness at 25° C., 40° C., and 75° C., of a Group of Antioxidants in Stabilizing Carotene Dissolved in Refined Cottonseed Oil; Effectiveness Based on Time Required for 20% and 50% Loss of Carotene.

Antioxidant	Stability Value			Antioxidant Index			Temperature Coefficient
	75° C.	40° C.	25° C.	75° C.	40° C.	25° C.	25° C./75° C.
	(hours)	*(hours)*	*(hours)*				
Control	4	100	400	1	1	1	100
TAP	11	100	400	3	1	1	36
Phospholipid	14	230	975	3	2	2	70
Hydroquinone	18	440	2125	5	4	5	118
PH	45	1125	7050	12	11	18	157
TPH	50	1350	7050	13	14	18	141
Antioxidant	Stability Value			Antioxidant Index			Temperature Coefficient
	75° C.	40° C.	25° C.	75° C.	40° C.	25° C.	25° C./75° C.
	(hours)	*(hours)*	*(hours)*				
Control	12	275	1200	1	1	1	100
TAP	36	520	2500	3	2	2	70
Phospholipid	31	560	2400	3	2	2	77
Hydroquinone	47	1475	4500	4	5	4	96
PH	195	3925	18500	16	14	15	95
TPH	189	3650	17500	16	13	15	93

antioxidants were added (Fig. 2) were nearly parallel to that of the control in most cases. Similar results were obtained with other antioxidants and in other oil carriers. This parallelism means that the temperature coefficients for rates of breakdown of carotene in the presence of added antioxidants are nearly the same as in their absence. Thus under the conditions of the tests it is possible to predict the approximate stability value at a given temperature of a carotene solution containing added antioxidants from a measured stability value at another temperature on the assumption that the temperature coefficient will be the same as that for the control (without added antioxidants).

The TEMPERATURE COEFFICIENTS (last column of Table I) represent the measured acceleration of carotene breakdown during storage at 75° C. over comparable 25° C. storage. For each antioxidant or combination this column presents the STABILITY $VALUE_{20}$ at 25° C. divided by the STABILITY $VALUE_{20}$ at 75° C. For the refined cottonseed oil control stored at 75° C., carotene breakdown was 100 times as rapid as in comparable 25° C. storage (Table I). For most of the more effective antioxidants studied their relative efficiencies were usually between the same and twice as high at the lower temperature as at the elevated temperature. For instance, at 25° C., PH and TPH were about one and one-half times as effective as the accelerated test would indicate (Table I). Therefore a prediction of the effectiveness of these antioxidant combinations at 25° C. based on the accelerated test observations would be a conservative estimate of their efficiency at the lower temperature.

TABLE II.

Relative Effectiveness at 25° C., and 75° C., of a Group of Antioxidants for Stabilizing Carotene Dissolved in Coconut Oil; Effectiveness Based on the Time Required for 20% Loss of Carotene.

Antioxidant	Stability $Value_{20}$		Antioxidant Index		Temperature Coefficient
	75° C.	25° C.	75° C.	25° C.	25°/75° C.
	(hours)	*(hours)*			
Control	1	86	1	1	86
Viobin	2	130	2	2	65
Phospholipid	3	170	3	2	57
Diphenylamine	5	280	5	3	56
TAP	12	97	12	1	8
alpha-Tocopherol	19	3538	19	41	186
Hydroquinone	36	5225	36	61	145
Nordihydroguaiaretic acid	46	7900	46	92	172
TCN	59	7650	59	89	130
VN	66	8750	66	102	133
TN	68	8150	68	95	120
PN	85	8875	85	103	104
PH	85	11875	85	138	140
TPN	86	8750	86	102	102
TCH	93	8900	93	104	96
TPH	118	11975	118	139	101

Carotene in Coconut Oil

Table II presents the STABILITY $VALUES_{20}$ for coconut oil solutions of carotene as affected by antioxidants. Also presented are the corresponding ANTIOXIDANT INDICES and the ratios of the STABILITY $VALUES_{20}$ at 25° C. to those at 75° C. for each antioxidant combination. Again as in refined cottonseed oil, the ANTIOXIDANT INDICES for the better antioxidants at 25° C. were at least as good as was predictable from the accelerated test. In almost all cases where protection was indicated by the test at 75° C. corresponding protection was found by lower-temperature storage. However, although general agreement was found in most instances, there was not a constant relationship

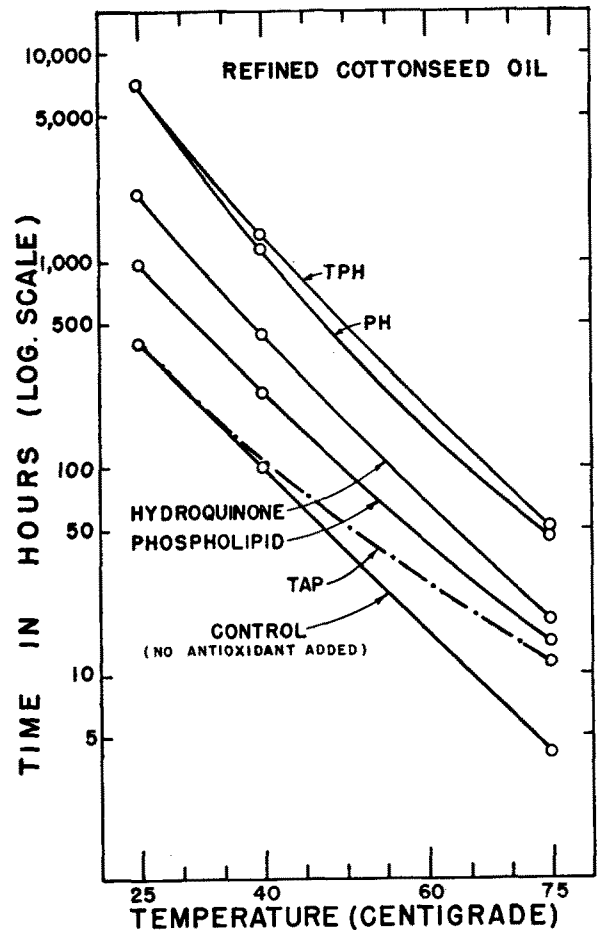

FIG. 2. Relationship of storage temperatures to STABILITY $VALUES_{20}$ for carotene dissolved in refined cottonseed oil, in the presence and in the absence of added antioxidants.

between the STABILITY $VALUES_{20}$ at the two temperatures. Again as in refined cottonseed oil, the combination TAP was relatively more effective at the higher temperature than at the lower temperature. Diphenylamine was also relatively less effective at 25° C. than at 75° C. Nordihydroguaiaretic acid and tocopherol were twice as effective at 25° C. as would have been predictable from data obtained with the accelerated test.

Nordihydroguaiaretic acid was more than twice as effective as tocopherol. The storage work at 25° C. confirms the previous report (3) on the effectiveness of nordihydroguaiaretic acid used either alone or in combination with acid-type synergists. In many instances the enhanced effectiveness of phenolic inhibitors due to the addition of the acidic-type inhibitor has not been so marked at 25° C. as it was at 75° C. Many of the antioxidant combinations containing nordihydroguaiaretic acid or hydroquinone yielded ANTIOXIDANT INDICES of 100 or more at 25° C. These materials increased the stability of the carotene based on the STABILITY $VALUE_{20}$ from less than four days without added inhibitor to well over a year by the addition of 0.05% of inhibitor.

Carotene in Lard

Table III presents the STABILITY $VALUES_{20}$ for carotene dissolved in lard without added antioxidants and in the presence of various added inhibitors. Although the STABILITY $VALUES_{20}$ in lard were lower than in

coconut oil for most antioxidant combinations, the ANTIOXIDANT INDICES for the better antioxidants were of the same order of magnitude as for coconut oil. Good predictions of stability at 25° C. were indicated if based on the STABILITY VALUES$_{20}$ at 75° C. The best antioxidants in the accelerated test again proved most effective in the experiments at 25° C. Thus with the accelerated test in a matter of hours or days it is possible to obtain an approximate estimate of the efficiency to be expected of an antioxidant at room temperature. Otherwise it would require months or years to determine the antioxidant efficiency by similar tests at the lower temperature.

Data presented in Table III show that ascorbic acid is more effective than l-ascorbyl palmitate as a synergist with tocopherol for stabilizing carotene in lard. Mattill (10) has recently suggested that, since many acid-type synergists are not fat soluble, adsorption must play a role in their action. He pointed out that phosphoric acid is more effective than cephalin as a synergist and suggested that if this consideration also applied to ascorbic acid, then the free acid should be more effective than its esters.

TABLE III.

Relative Effectiveness at 25° C. and 75° C. of a Group of Antioxidants for Stabilizing Carotene Added to Lard; Effectiveness Based on the Time Required for 20% Loss of Carotene.

Antioxidant	Stability Value$_{20}$		Antioxidant Index		Temperature Coefficient
	75° C.	25° C.	75° C.	25° C.	25°/75° C.
	(hours)	*(hours)*			
Control	⅔	49	1	1	74
Viobin	1½	43	3	1	29
5x Viobin	4	46	6	1	12
Diphenylamine	5	50	8	1	10
alpha-Tocopherol	9	1040	13	21	116
AT	12	165	18	3	14
TAP	20	120	30	2	6
TCN	22	2750	33	56	125
Hydroquinone	24	2050	36	42	85
AsT	28	925	42	19	33
TPC	33	2100	50	43	64
TPN	59	5500	89	112	93
TPH	108	6000	162	122	56

Effect of Ratio of Exposed Surface to Volume

The conditions of storage at 25° C. in the experiments reported above were not designed to be the most ideal from the standpoint of preserving the provitamin. Rather, conditions were the same as for the accelerated test so that the effect of temperature could be properly evaluated. For instance, with a one-gram sample of oil a relatively thin layer of oil was exposed to the air. Under commercial storage conditions containers are usually completely filled or nearly so. In order to compare the conditions of our test with more common shelf-storage conditions some larger samples were prepared. Storage tests at both 25° C. and 75° C. were made on 16-gram samples of carotene-in-oil solutions prepared with and without added antioxidants. Samples were stored in the same type of vials and lightly stoppered with cotton plugs as in the previously described tests. In these deep samples fading was most rapid in the upper portion. In order to obtain representative samples for analysis solutions were uniformly mixed prior to removal of a portion for analysis.

Data obtained from these deep samples are presented in Table IV. In the relative absence of antioxidants, either added or naturally present, the effect of depth of layer was very marked. Thus carotene was about 10 times as stable in the 16-gram lard and coconut oil controls as in the corresponding 1-gram samples. In the cacao butter control, which contained natural antioxidants, the effect of the depth of layer of oil was not nearly as marked as in the oils which were relatively deficient in natural antioxidants (cf. Ref. 3). In the 16-gram samples, to which an effective antioxidant combination (PH) was added, carotene stability was very similar in the various oils (Table IV) and the difference in carotene stability between the 1-gram and 16-gram samples was not as marked as in the controls (Tables II, III, IV, cf. Ref. 3).

TABLE IV.

Relative Effectiveness of Antioxidants at 25° C., and 75° C. for Stabilizing Carotene Dissolved in Cacao Butter, Lard, and Coconut Oil. Storage Samples Consisted of 16 Grams of the Oil Solutions, and Effectiveness Was Based on the Time Required for 20 Per Cent Loss of Carotene.

Antioxidant	Stability Value$_{20}$		Antoxidant Index		Temperature Coefficient
	75° C.	25° C.	75° C.	25° C.	25°/75° C.
	(hours)	*(hours)*			
		Lard			
Control	10	1125	1	1	113
Hydroquinone	85	4400	9	4	52
PH	160	12750	16	11	80
		Coconut Oil			
Control	12	320	1	1	27
Hydroquinone	65	9600	5	30	148
PH	168	14500	14	45	86
		Cacao Butter			
Control	60	2360	1	1	4
PH	165	12600	3	6	8

Summary

An attempt has been made to evaluate the elevated temperature test used for carotene stability studies in this laboratory. The stability of carotene in edible oils containing various added antioxidants was determined by storage at 25° C. and in some cases 40° C. and compared with stability as determined by the accelerated test at 75° C. In general, the results obtained at 75° C. were in agreement with those obtained at 40° and 25° C. With most of the better antioxidants the protection found at the lower temperature was better than that indicated by the rapid test.

Solutions of carotene in edible oils, which ordinarily might lose a significant amount of carotene in a week or less at 25° C., can be protected by addition of antioxidants, and protection can be afforded for periods well over a year without such special precautions as refrigeration or storage under nitrogen or in evacuated containers. The effectiveness of nordihydroguaiaretic acid as an antioxidant for carotene has been confirmed by room-temperature storage tests.

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