Stabilization of Carotene With Nordihydroguaiaretic Acid and Other Antioxidants

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EDIBLE oils fortified with carotene can be of value in supplementing our present restricted supply of vitamin A. Such fortification, however, necessitates attention to stabilization since carotene may be readily lost by oxidation. Although added antioxidants have long been successfully employed to protect carotene in inert organic solvents, their value in edible oil solutions of carotene has not been well established. Some workers have believed that added antioxidants would not enhance the effectiveness of the natural antioxidants of the oils (2, 14). A recent publication from this Laboratory has shown, however, that by the addition of several different antioxidants together carotene stability can be enhanced as much as 20-fold, even in vegetable oils that already contain natural antioxidants (22).

Many substances have been suggested as antioxidants for the stabilization of edible oils. Their relative effectiveness in stabilizing carotene cannot, however, be determined from work on rancidity, since, as is well known, antioxidants vary in effectiveness with the substrate (10). This paper presents the results of a study of the effectiveness of a group of antioxidants for carotene in edible oil solutions. Several of the antioxidants studied have already been shown to be of value in stabilizing edible oils. Since stability of carotene varies with the solvent, four oils were used as carriers. They included refined coconut and cottonseed oils, cacao butter, and lard.

Experimental Methods

The effectiveness of the antioxidants as stabilizers for carotene was determined by a controlled accelerated test, previously described (22). The antioxidants were tested individually and in various combinations. Each was incorporated in the proportion of 0.5 mg. and carotene in the proportion of 1.2 mg. per gram of oil. The storage temperature was 75°C. A chromatographic adsorption method (3) was employed for the analysis of carotene in the oil.

Data on the stability of carotene, as affected by both carriers and added antioxidants, are summarized in Table I. Some of the data previously obtained with cottonseed oil (22) are included for comparison. Pyrogallol (not accepted as edible) has been shown to be a powerful antioxidant for carotene (17) and was included to serve as a standard.

Phosphorus determinations on the oils permitted computations of the phospholipid contents. The oils were also analyzed for tocopherol by the Parker-McFarlane modification of the Emmerie-Engel method (16). Initial acid and peroxide numbers were determined on all the oils. Peroxides were determined according to the method of Wheeler (20), peroxide number being the number of millimoles of peroxide per 1,000 grams of oil. The data are presented in Table II.

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TABLE II
Constants of Oils

	Coconut Oil	Cottonseed Oil	Cacao Butter	Lard
Acid number [1]	0.21	0.09	1.88	0.84
Iodine number (Hanus)	7.9	111.0	35.8	68.0
Original peroxide number	3.49	2.49	0.00	1.11
Phospholipids [2]	0.002%	0.002%	0.057%	0.003%
Tocopherols	0.004%	0.036%	0.012%	0.001%

[1] Mg. KOH per gm. oil.
[2] Approximation, calculated as percent glyceryl phosphatides ($P_2O_5 \times 11.37$).

For many of the antioxidant combinations, the decrease of carotene in the oil was followed until complete or nearly complete loss occurred, and at intervals samples were analyzed for peroxide as well as carotene content. A large number of storage samples of the different oils containing the various added antioxidants were thus analyzed, and by plotting carotene loss versus peroxide content the curves in Figure 1 were obtained.

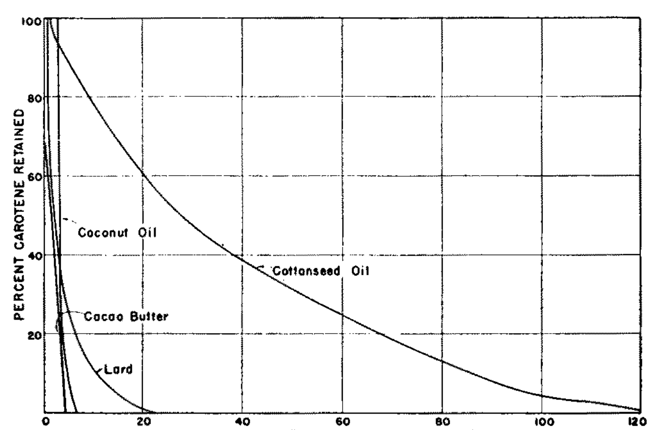

FIG. 1. Relation between Peroxide Number and Carotene Loss at 75°C.

Relative Effectiveness of Oils in Stabilizing Carotene

IN THE absence of added antioxidants, carotene was more stable in refined cottonseed oil, which naturally contains alpha-tocopherol, than in coconut oil or in lard, which are relatively deficient in this antioxidant (Tables I and II). Carotene was even more stable in cacao butter than in cottonseed oil, although cottonseed oil contains a larger amount of natural tocopherol. This result was undoubtedly partly due to the fact that the cacao butter contains considerably less unsaturated fatty acid, which contributes to the decreased susceptibility of the cacao butter to oxidation (Table II). Another factor which might contribute to the greater stability of carotene in cacao butter, as compared with cottonseed oil, is the presence of about 0.06 percent of phospholipid (Table II) which could stabilize and enhance the antioxidant effect of the tocopherol naturally present (19).

Carotene was three times as stable in coconut oil containing added tocopherol as in a comparable solution of cottonseed oil with or without added tocopherol. Addition of phospholipid with tocopherol enhanced the stability of carotene in both oils, but the stability of carotene in the coconut oil was still three times that of carotene in the cottonseed oil. These comparisons show that increased unsaturation of an oil tends toward decreased effectiveness of alpha-tocopherol in stabilizing carotene. Similarly, Bailey *et al.* (1) found that by reducing the degree of unsaturation of peanut oil by hydrogenation, they could increase the antioxidant effect of tocopherol.

Effectiveness of Antioxidants in Stabilizing Carotene

When carotene and one or more antioxidants are added to an oil containing natural antioxidants and unsaturated fatty acids, a very complex system is formed. The following interactions may occur: Autoxidation of the carotene may be hastened by oxidation of the double bonds of unsaturated fatty acids or, conversely, the carotene may act as a pro-oxidant to hasten the autoxidation of the fatty acids. Both the carotene and the fatty acids may be destroyed by direct oxidation. The naturally present or added antioxidants may act with different degrees of effectiveness toward either the carotene or the fatty acids. The effects observed may be due to interaction between naturally present and added inhibitors. The system thus becomes difficult to explain; therefore values for the stability of carotene will be presented here with little attempt at interpretation.

Nordihydroguaiaretic acid (NDGA) has been shown to be effective in increasing the storage life of lard (12). The combination of NDGA and citric acid showed a marked synergism in stabilizing cottonseed oil (13). Our data show that NDGA is one of the more promising antioxidants for stabilizing carotene in various oil solutions, and that its effectiveness was considerably enhanced when it was used in combination with cottonseed phospholipid or citric acid. Addition of tocopherol to the combination of citric acid and NDGA increased their value as antioxidants in lard. The NDGA is of plant origin, and has the approval of the War Food Administration for incorporation into lard or rendered pork fat in a quantity not exceeding 0.01 percent (Meat Inspection Memorandum No. 25, dated December 11, 1943).

In general, hydroquinone was as effective as NDGA when used either alone or in combination with acid-type inhibitors. It is less fat-soluble than NDGA, however, and is not accepted for use in foods by the Council of Pharmacy of the American Medical Association (5).

Alpha-tocopherol was less effective than NDGA when added to the various oil solutions of carotene. Carotene was not further stabilized by addition of alpha-tocopherol in the two oils which naturally contain 0.01 percent or more of tocopherol. Effectiveness of tocopherol was enhanced by combination with acid-type inhibitors. Aside from its antioxidant effectiveness *in vitro*, however, it would appear desirable to incorporate alpha-tocopherol into carotene-in-oil solutions which are naturally deficient in this substance because of its remarkable sparing effect on carotene *in vivo* (8).

TABLE I

Time for 20 Percent Loss of Carotene in the Various Oils as Influenced by Antioxidants Added Individually or in Combination,[1] Samples Stored at 75°C.

Oil	Antioxidants (0.5 mg. of each per gram of oil)											
	None	T	H	N	G	TH	TN	TG	P	D	V	VN
	hrs.	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*
Coconut	1	20	23	40	39	28	65	....	75	5	3	49
Lard	⅔	8	24	26	25	....	26	....	90	4½	1½	45
Cottonseed	5	6	25	19	43	27	24	....	60	7	14	40
Cacao butter	33	27	98	77	77	93	56	....	80	35	38	62
and Cottonseed Phospholipid (0.5 mg. per gram of oil)												
Coconut	3	59	75	76	40	84	82	76	....	....	....	....
Lard	1½	25	81	48	30	108	62	76	....	....	....	....
Cottonseed	17	18	73	70	82	80	74	67	....	....	....	....
Cacao butter	43	43	95	73	68	97	69	78	....	....	....	....
and Citric Acid (0.5 mg. per gram of oil)												
Coconut	2	39	84	50	....	87	61	....	....	....	....	....
Lard	2	22	62	50	....	112	112	....	....	....	....	....
Cottonseed	12	16	54	72	....	53	63	....	....	....	....	....
Cacao butter	68	84	100	101	....	112	112	....	....	....	....	....
and Ascorbic Acid (0.5 mg. per gram of oil)												
Coconut	4½	15	13	14	....	....	....	....	....	....	....	....
Lard	2	28	22	18	....	....	....	....	....	....	....	....
Cottonseed	15	17	40	45	....	....	....	....	....	....	....	....
Cacao butter	65	85	91	86	....	....	....	....	....	....	....	....
and l-Ascorbyl Palmitate (0.5 mg. per gram of oil)												
Coconut	3	11	11	11	....	14	....	....	....	....	....	....
Lard	2	12	13	15	....	19	....	....	....	....	....	....
Cottonseed	13	11	15	39	....	13	....	....	....	....	....	....
Cacao butter	31	48	51	54	....	73	....	....	....	....	....	....
and Citric Acid and Phospholipid (0.5 mg. of each per gram of oil)												
Coconut	3½	35	....	....	....	....	....	....	....	....	....	....
Lard	5	33	....	....	....	....	....	....	....	....	....	....
Cottonseed	15	20	....	....	....	....	....	....	....	....	....	....
Cacao butter	81	85	....	....	....	....	....	....	....	....	....	....
and l-Ascorbyl Palmitate and Phospholipid (0.5 mg. of each per gram of oil)												
Coconut	2½	13	9	....	....	....	....	....	....	....	....	....
Lard	2	20	....	....	....	....	....	....	....	....	....	....
Cottonseed	12	16	19	....	....	....	....	....	....	....	....	....
Cacao butter	40	66	67	....	....	....	....	....	....	....	....	....
and Maleic Acid (0.5 mg. per gram of oil)												
Cottonseed	6	....	20	15	....	....	....	....	....	....	....	....

[1] For example, the time in hours for 20 percent carotene loss at 75°C. when alpha tocopherol, hydroquinone, and citric acid are added to coconut oil is 87.

Symbols used for antioxidants: T—Alpha Tocopherol; H—Hydroquinone; N—Nordihydroguaiaretic Acid; G—Gallic Acid; P—Pyrogallol; D—Diphenylamine; V—VioBin Antioxidant.

Gallic acid was about equal to NDGA as an antioxidant for carotene when added to the various oils. When used in combination with phospholipid its effectiveness was enhanced in the cottonseed oil but not in the other three oils. Gallic acid is not very soluble in oil and even the small quantity added as an antioxidant failed to go into solution entirely. Hilditch (10) reported ethyl gallate to have relatively low efficiency for carotene in ethyl acetate solution but high potency as an antioxidant for the unsaturated glycerides of butterfat and for vitamin A in halibut-liver oil or margarine.

l-Ascorbyl palmitate (palmityl ascorbic acid) is an effective antioxidant for stabilizing lard when used in combination with tocopherol and phospholipid (18). It has recently been shown that l-ascorbyl palmitate, when fed together with tocopherol and carotene to rats, enhances the vitamin-A-sparing action of the tocopherol (9). In our studies, this antioxidant caused a 3-fold increase in stability of carotene in lard, cottonseed oil, and coconut oil. No further enhancement was obtained when it was added in combination with phospholipid and tocopherol to these oils. However, in the cacao butter the combination of tocopherol, ascorbyl ester, and phospholipid caused a significant increase in the stability of the carotene. Our previous work showed this combination of antioxidants to be effective in stabilizing carotene in mineral oil but not in cottonseed oil (22).

VioBin antioxidant, which has been shown to give a 2- to 3-fold increase in the stability of lard or vegetable oil (11), also caused only a 2- to 3-fold increase in the stability of carotene dissolved in coconut oil, cottonseed oil, and lard and was ineffective in cacao butter. Even when used in concentrations as high as 1 percent, it was only slightly effective as compared with NDGA under the conditions of our test.

Ascorbic acid inhibits the development of rancidity and loss of vitamins A and E in emulsified fats when dissolved in the aqueous phase of the emulsion (7). In our test, however, ascorbic acid, which is not very soluble in oils, was only slightly effective in stabilizing carotene in the various oils.

Cottonseed phospholipid (15) caused about a 3-fold increase in the stability of the added carotene in coconut oil, cottonseed oil, and lard (Table I). The addition of phospholipid to the cacao butter caused an enhancement in stability of added carotene, although its relative effectiveness in this oil could not be determined since about 0.06 percent of phospholipid was already present in this oil (Table II). Phospholipid is very effective in enhancing the antioxidant effect of phenolic-type inhibitors (Table I). Citric acid and other acidic-type inhibitors tested acted much like phospholipid. Maleic acid, however, was ineffective for stabilizing carotene in cottonseed oil.

Guaiacol, eugenol, vanillin, vanillyl alcohol, and vanillal acetone exhibited almost no antioxidant effect toward carotene when tested individually in cottonseed oil solution. When these antioxidants were tested in combination with phospholipid the antioxidant effect was only slightly better than that of phospholipid alone.

Diphenylamine, previously shown to be an efficient antioxidant for carotene in pelleted mixtures prepared with mineral oil (4, 21), produced about a 5-fold increase in stability of carotene in coconut oil and a 7-fold increase in stability of carotene in lard, but was not effective in cacao butter or cottonseed oil. Diphenylamine, like alpha-tocopherol, appears to be most effective in an inert solvent.

Peroxide Accumulation and Carotene Loss

Gould *et al.* (6) recently reported a study of the relationship of the stability of carotenoids of butter and butter oil to formation of peroxides. In butter, peroxide formation greatly preceded any detectable loss of carotenoids, whereas in butter oil, oxidized under controlled accelerated conditions, destruction of carotenoids was great in comparison to peroxide changes. Approximately 50 percent of the carotenoid was destroyed before a peroxide value of 1.5 was reached. Our results with lard, cacao butter, and coconut oil, under the accelerated conditions of our test, are similar to those obtained with butter oil by Gould (Figure 1). There appears to be no relationship between the accumulation of peroxides and loss of carotene in these oil solutions.

In cottonseed oil (22), accumulation of peroxides and loss of carotene proceed simultaneously and begin with storage. The carotene is destroyed at much higher peroxide numbers than in the other oils and a much higher peroxide number has been reached when the carotene becomes completely decolorized. High peroxide numbers may be related to high linoleic acid content. Cottonseed oil contains about 40 percent of linoleic acid, whereas other oils studied have about 2 percent or less of this acid. The peroxide numbers at which carotene has virtually disappeared in lard and cottonseed oil, about 20 and 120, respectively (Figure 1), are almost exactly the average peroxide numbers corresponding to the end of the induction period in the Swift stability test. It thus appears that loss of carotene may be related to destruction of naturally present or added antioxidants during the course of the induction period, rather than to the development of peroxides. It is commonly observed that in the course of stability tests on vegetable oils there is a marked decrease in the color of the oil (in part due to the destruction of carotenoid pigments) coincident with the development of marked rancidity. Previous studies with various fish-liver oils show that vitamin A loss often occurs concurrently with the development of rancidity, as measured by increase in peroxide number of the oil.

Conclusions

The practicability of stabilizing carotene in animal and vegetable oils with antioxidant and thus reducing the loss in storage of the provitamin has been demonstrated, and should stimulate increased interest in this means of supplementing the present restricted supply of vitamin A. Carotene was very stable in cacao butter and its incorporation in concentrated rations such as chocolate bars made with cacao butter might afford a satisfactory way of supplying provitamin A to the diet. Other methods that readily suggest themselves include its addition to cheese, butter, margarine, peanut butter, other spreads, and salad dressings.

Summary

Relative values for the carotene-stabilizing effects in edible oil solutions of a number of antioxidants, alone and in various combinations, have been determined and are presented. One of the more promising

for this purpose is nordihydroguaiaretic acid. When this antioxidant is used together with phospholipid or citric acid, its effectiveness is enhanced. Addition of alpha-tocopherol to oils containing only traces of this antioxidant causes a significant increase in carotene stability, and its sparing effect on carotene *in vivo* (8) would further add to the desirability of its use in such oil solutions of carotene.

In refined cottonseed oil, carotene destruction occurred concurrently with the accumulation of peroxides. In the more saturated oils, carotene destruction occurred before much peroxide was detected in the oils.

Acknowledgments

The authors are indebted to L. M. White of this Laboratory for the phosphorus determinations on the various oils, and to H. S. Olcott, also of this Laboratory, for the preparation of the cottonseed phospholipid. They are also indebted to the Wm. J. Stange Company for the nordihydroguaiaretic acid; to the Eastern Regional Research Laboratory for the l-ascorbyl palmitate; to Swift and Company for the prime steam-rendered lard; and to El Dorado Oil Company for the coconut oil used in the experiments described in this paper.

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Synthetic Resins and the Drying Oils[1]

Some Aspects of Their Interrelation

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THE origin of the art of combining drying oils with resins appears to be lost in antiquity. Both components were natural products which were very little changed prior to use. Then, as later, it was recognized that films of hard resin were lacking in toughness and in flexibility and also that films of drying oils alone could be improved in brilliance and in the rapidity with which useful hardness was developed through the inclusion of resin.

While considerable improvement was made in the crushing and refining of the drying oils and in their heat processing, there was no basic change in the heat processing of the various oils and resins until recently. True, varnishes of widely different characteristics were obtained, but it may be argued that these characteristics were the rather mechanical averaging-out of the properties of the ingredients. It was not until the advent of the synthetic resins that in the field of air-drying coatings it became possible to add two and two and come out with five for an answer.

First came the rosin modified phenol-aldehyde resins. These, when properly combined with drying oils, gave a new order of speed to air-drying varnishes. When tung oil was employed, a useful increase in water resistance and chemical resistance was obtained. Certain types of these resins yielded varnishes with far less than the usual tendency to soften or sweat-back with age, a trying property shared by many of the natural oils and a few of the newer "synthetic" products. Following these rosin modified types came the 100% synthetic phenol-aldehyde resins. Many types were soon developed showing a very wide divergence as to their effect on the bodying rate of drying oils. The investigation of these differences and the comparison of the various types of phenolic resins against other classes makes a fascinating study. Unfortunately, it is far too involved a subject to consider here. Turkington and Allen (4) have described in detail the resin properties obtained through the use of the various substituted phenols. Shuey (2) compared a number of the phenol-aldehyde resins with other types in terms of their behavior with the various drying oils. In particular, the discussion bearing on the decreased oxygen intake of varnish films based on certain phenolic resins, the mechanism of the polymerization of these resins with oils and the effect of heat treatment and resin selection on both the application and service properties of the terminal varnishes merit close attention. In effect, the resin properties were added to the oil properties with a resultant good somewhat beyond their simple sum. This work, and that which will undoubtedly follow it, should give the oil chemist additional clues

[1] This paper was presented in the Drying Oils Symposium before the American Oil Chemists Society, Chicago, Illinois, October 26, 1944.