

(6) on a comparable substrate. With the vegetable fats, however, it was not so effective but was quite consistent. Ascorbyl palmitate was erratic in its effect; at times its index was as high as that of NDGA, but not always. Similar antioxidant indices for ascorbyl palmitate in various fat substrates were obtained by Riemenschneider *et al.* using an oven test at 100° (7). Greatest effectiveness as an antioxidant was shown by gallic acid.

With each of these antioxidants, as with most of the others that were used, uniform dispersion and means of suspension were found to be a serious problem. Even at the lowest concentrations used (0.02%) none of the antioxidants were completely miscible with the fats. They remained in a state of gross suspension and settled out in an oil or a melted fat. Greatest difficulty in this respect was encountered with gallic acid. It is obvious, therefore, that the value of these compounds as antioxidants would be limited if the substrate were to be maintained for any length of time at a temperature higher than its melting point; the antioxidant would then simply settle to the bottom of the melted fat.

Ascorbic acid, which exhibited an index of the same order as NDGA, showed the greatest tendency to remain in suspension.

None of the other substances used showed any particular promise as antioxidants.

Synergism was negative in several of the combinations tried but was quite effective when NDGA was

used in conjunction with either phosphoric or citric acid. It was observed that extensive charring occurred under the conditions of the test when the concentration of phosphoric acid was increased above 0.05%. On the other hand, it should be noted that the addition of only 0.01% phosphoric acid was necessary to produce an index of 4.7 with 0.05% NDGA.

### Summary

1. A number of compounds and combinations of compounds have been examined for antioxidant properties.

2. The most effective single compound was gallic acid, although NDGA, ascorbic acid and ascorbyl palmitate each about doubled the keeping time of any given vegetable fat or oil.

3. Amino acids were negative with the exception of methionine.

4. NDGA in combination with either citric acid or phosphoric acid showed marked synergism. These combinations were very effective antioxidants.

### BIBLIOGRAPHY

1. King, A. E., Roschen, H. L., and Irwin, W. H., *Oil and Soap*, 10, 105 (1933).
2. Mehlenbacher, V. C., *Oil and Soap*, 19, 137 (1942).
3. Wheeler, D. H., *Oil and Soap*, 9, 89 (1932).
4. Freyer, E., *Oil and Soap*, 13, 227 (1936).
5. Swern, D., Sterton, A. J., Turer, J., and Wells, P. A., *Oil and Soap*, 20, 224 (1943).
6. Lundberg, W. O., Halvorson, H. O., and Burr, G. O., *Oil and Soap*, 21, 33 (1944).
7. Riemenschneider, R. W., Turer, J., Wells, R. A., and Ault, W. O., *Oil and Soap*, 21, 47 (1944).

## Effect of Antioxidants, Individually and in Combination, on Stability of Carotene in Cottonseed Oil

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Carotene in oil solution is becoming increasingly important as a source of vitamin A. In work reported in 1933 Baumann and Steenbock (2) found that refined cottonseed oil was outstanding among the common edible oils as a stabilizing solvent for carotene. Despite the presence of natural antioxidants in cottonseed oil (10), however, loss of carotene from a solution occurs under normal storage conditions. Even in the presence of hydroquinone which is used extensively as a stabilizer, appreciable loss occurs and a more efficient stabilizing agent would be useful. In an effort to find such a stabilizer, a systematic study of additional antioxidants for carotene has been made in this laboratory.

Olcott and Mattill (9) first demonstrated synergistic action with combinations of edible antioxidants. Thus, cephalin greatly enhances the antioxygenic activity of tocopherol in autoxidizing fats (9, 13). Ascorbic acid in combination with tocopherol is effective in stabilizing lard (4). Lecithin in combination with hydroquinone is effective in stabilizing vitamin

A in fish liver oils (6). Recently workers at the Eastern Regional Research Laboratory have shown that ascorbic acid esters such as l-ascorbyl palmitate, when added together with lecithin and tocopherol to lard, cause a significant increase in stability of the lard (12).

The success obtained with combinations of antioxidants in stabilizing other autoxidizing systems suggests that combinations might also be effective for stabilizing carotene in refined cottonseed oil. The following antioxidants were studied: cottonseed phospholipid (8), soybean phospholipid, alpha-tocopherol, l-ascorbyl palmitate, and hydroquinone. These antioxidants were used individually and in various combinations. The relative effectiveness of the antioxidants for carotene was determined both in a highly refined medicinal mineral oil and in refined cottonseed oil (Wesson). The whole question of the action of antioxidants is complicated by the fact that we now recognize at least two methods by which they exert their protective power. Thus they may act to prevent simple atmospheric oxidation or they may act to inhibit coupled reactions involving the formation of oil peroxides and the resultant destruction of

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carotene. Stability studies of carotene in mineral oil were included to determine the direct effect of the antioxidants in preventing simple atmospheric oxidation, since peroxides do not accumulate in these solutions until the carotene has become decolorized.

### Accelerated Test

Commercial crystalline carotene, repurified by precipitation from chloroform with methyl alcohol (1) was dissolved in the mineral or refined cottonseed oil and adjusted to a concentration of 1.2 mg. per gram of oil. A series of one-gram samples of carotene-in-oil solution were weighed into homeopathic vials<sup>1</sup> (21 mm. in diameter by 85 mm. in height) thus exposing a comparatively thin layer to oxidation. The antioxidants in peroxide-free ether solution were then added and the vials gently rotated in order to distribute the antioxidants uniformly in the oil. The antioxidants were added at the rate of 0.5 mg. per gram of oil and when more than one antioxidant was used, 0.5 mg. of each was added, except as otherwise noted. To compare the relative effects of the antioxidants in stabilizing the oil, comparable samples without added carotene were prepared. To hasten the decomposition of the carotene, which is relatively slow at room temperature in refined cottonseed oil, the tests were made at 75° C. A method of this kind must be used when results are required quickly, and experience has shown that data thus obtained usually furnish a useful guide to behavior during storage at lower temperature. The vials left open for free circulation of air were placed in a constant temperature oven. The ether, which amounted to from 0.05 to 0.15 ml., soon evaporated at the elevated temperature of the test. At intervals two or more of the vials were removed for analysis. Both carotene and peroxide were determined. The carotene was determined by a previously described method (3). Peroxides were determined according to the method of Wheeler (14). The peroxide number is given as the number of millimoles of peroxide per 1,000 grams of oil.

The times required for 20 and 50 per cent destruction of carotene were chosen as reference points for certain comparisons. The 20 per cent point was chosen because it represented a significant loss of carotene as well as an adequate time interval for measurement. The 50 per cent point was chosen because certain antioxidants permitted the rapid loss of carotene during the early part of the storage period but were relatively more effective over longer storage periods. The loss of carotene was followed until complete or nearly complete destruction of carotene took place (Figs. 1 and 2).

### Effect of Antioxidants

In this report the necessity for repeatedly referring to the various antioxidants and their combinations makes it desirable to abbreviate them. Accordingly the following symbols are used to denote the antioxidants:

T—alpha tocopherol  
A—l-ascorbyl palmitate  
P—phospholipid  
H—hydroquinone

and for example: TAP—alpha tocopherol, l-ascorbyl palmitate, and phospholipid used in combination.

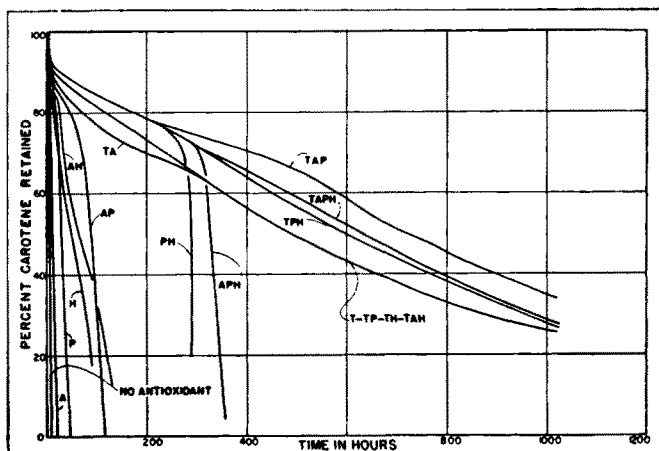


Fig. 1. Effect of antioxidants on the rate of loss of carotene in mineral oil. (75° C.)

The data (Fig. 1) confirm previously reported results (7) indicating that T is very effective in stabilizing carotene in mineral oil. P, H, A and the combinations AP and AH were only slightly effective as compared to T. TAP, TAPH and TPH gave slightly greater protection than T alone. The other combinations containing T showed protective power very similar to T alone. PH and APH were as effective as TPH and TAPH until about 25 per cent of the carotene was lost. Then the samples containing PH and APH lost their carotene very rapidly, whereas those containing TPH and TAPH continued the slow rate of carotene loss over an extended period.

Peroxides were not found in the mineral oil solutions until the carotene was destroyed. After all of the carotene color had disappeared, peroxides formed in the mineral oil and reached high values. The method of determining peroxide numbers did not permit differentiation of carotene peroxides from those formed by mineral oil oxidation.

In refined cottonseed oil solution 20 per cent of the carotene was destroyed in five hours when no antioxidants were added (Table I). This is about twice the time required in mineral oil and affords a measure of the effectiveness of the natural antioxidants present in the refined cottonseed oil.

The effectiveness of the added antioxidants in the refined cottonseed oil was quite different from that in mineral oil (Figs. 1 and 2). Addition of 0.05 per cent of tocopherol did not enhance the effectiveness of the tocopherol or other antioxidants naturally present in the refined cottonseed oil. Golumbic (5) has shown that above a certain concentration further additions of tocopherol are decreasingly effective. Each of the other three antioxidants (A, P, H) when added individually caused a 3- to 4-fold increase in the stability of the carotene as measured at the 20 per cent point. Soybean phospholipid was found to be as effective as cottonseed phospholipid when used alone or in various combinations.

At the 20 per cent point PH was very effective in refined cottonseed oil, producing a 15- to 20-fold increase in the stability of the carotene. At this point the other binary combinations were no more effective than the individual antioxidants acting alone. TH, which is very effective in mineral oil and which was shown to be effective in ethyl linoleate (11), was also moderately effective in refined cottonseed oil (Fig. 2). Of the four possible ternary combinations, TPH was

<sup>1</sup>The reuse of vials cleaned by the usual methods gave rise to erratic results. Since the vials are inexpensive, only new vials which had been rinsed with distilled water, dried and thoroughly washed with petroleum ether were used.

TABLE I

Time for 20 and 50 Per Cent Loss of Carotene in Mineral and Refined Cottonseed Oil, Together With the Peroxide Number of the Refined Cottonseed Oil With and Without Carotene. Samples Stored at 75° C.

Antioxidant <sup>1</sup>	Time for 20% loss of carotene		Peroxide number <sup>2</sup> of cottonseed oil at time of 20% carotene loss		Time for 50% loss of carotene		Peroxide number <sup>2</sup> of cottonseed oil at time of 50% carotene loss	
	Mineral oil	Cottonseed oil	Oil with carotene	Oil without carotene	Mineral oil	Cottonseed oil	Oil with carotene	Oil without carotene
	hours	hours	mM/kg. oil	mM/kg. oil	hours	hours	mM/kg. oil	mM/kg. oil
None.....	2	5	14.5	24.0	3	25	46.0	46.0
T.....	120	5	23.5	28.5	488	27	61.0	58.0
A.....	5	12	7.5	5.0	10	32	36.0	30.0
P.....	20	17	16.0	17.5	28	35	41.5	35.5
H.....	12	19	10.5	8.0	48	115	38.0	30.0
TA.....	72	14	11.5	6.5	488	36	44.0	40.0
TP.....	120	17	23.5	19.5	488	40	62.0	60.0
TH.....	120	16	15.0	14.0	488	90	61.0	70.0
AP.....	46	13	12.0	6.0	88	32	35.0	27.0
AH.....	24	16	4.0	5.0	62	220	29.0	26.0
PH.....	175	80	9.0	.....	288	228	27.5	.....
TAP.....	175	16	17.5	10.0	720	48	56.5	40.0
TAH.....	120	15	6.5	6.0	488	218	48.0	37.5
APH.....	175	18	4.0	4.5	325	220	28.0	23.0
TPH.....	175	80	9.0	.....	596	228	28.0	.....
TAPH.....	175	16	5.0	5.0	630	218	45.5	35.0

<sup>1</sup>T, alpha-tocopherol; A, l-ascorbyl palmitate; P, phospholipids from cottonseed; H, hydroquinone.

<sup>2</sup>Millimoles per kilogram of oil.

the best at the 20 per cent point. However, it was no more effective than the binary combination PH, possibly because sufficient T was already present in the oil. It is possible that the PH stabilized the tocopherol, which was then able to protect the carotene. It has been shown that cephalin (a constituent of the phospholipids) (13) and hydroquinone (11) tend to stabilize and enhance the antioxidant activity of tocopherol (9). TAPH showed only a 3- to 4-fold increase in carotene stability at the 20 per cent point.

In following the destruction of carotene beyond the 20 per cent point it was found that APH, TAH, AH and TAPH were almost identical in causing a marked retardation in the rate of carotene destruction (Fig. 2). Their effectiveness at the 50 per cent point was practically equal to that of the combinations PH and TPH.

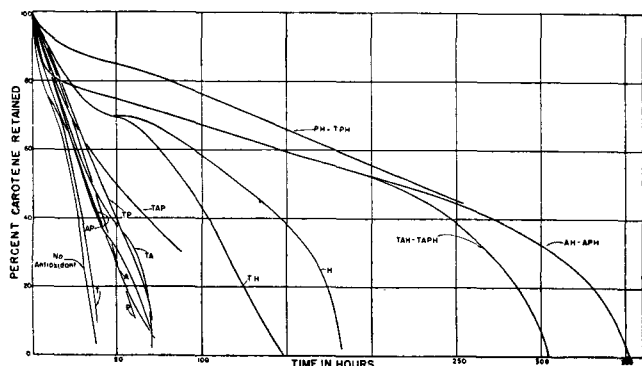


FIG. 2. Effect of antioxidants on the rate of loss of carotene in refined cottonseed oil. (75° C.)

The antioxidants that stabilized the carotene to the greatest extent were also the most efficient in stabilizing the oil (Table I). The formation of peroxide occurs concurrently with the destruction of carotene. As an example, Figure 3 shows the relation of carotene destruction to peroxide formation in solutions containing the added antioxidants PH and TPH.

Carotene did not affect the formation of peroxides in the refined cottonseed oil until the carotene had become almost completely oxidized. As the carotene completely disappeared, the peroxide formation was greatly accelerated. For example, after 111 hours a sample of refined cottonseed oil containing carotene

and TH retained 33 per cent of the original carotene and had a peroxide number of 90. A comparable storage sample of oil containing TH (and no carotene) had the same peroxide number. However, after 161 hours the carotene had disappeared and the peroxide number in this sample had gone up to 275 while in the comparable sample containing TH (and no carotene) the peroxide had reached a value of but 120. All other cases examined behaved similarly.

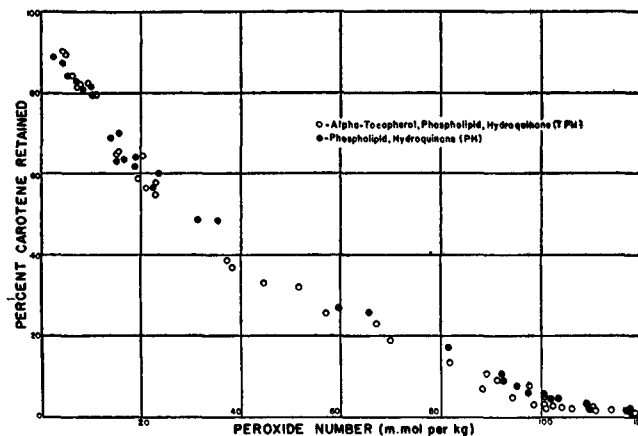


FIG. 3. Relation between carotene destruction and peroxide formation in refined cottonseed oil. (75° C.)

The effectiveness of the antioxidant combination PH for stabilizing carotene in refined cottonseed oil was determined over a wide range of concentrations and varying ratios of phospholipid to hydroquinone (Table II). The addition of 0.05 per cent P and 0.01 per cent H increased the time required for 20 per cent destruction of carotene from 5 hours (without added antioxidants) to 56 hours. A further 3-fold increase in stability (148 hours) was obtained by a 100-fold increase in antioxidant concentration (5 per cent P and 1 per cent H).

### Summary and Conclusions

By an accelerated test, l-ascorbyl palmitate, alpha-tocopherol, hydroquinone, and phospholipids from cottonseed and soybean oil have been evaluated singly and in combinations, for their antioxidant effect on carotene in mineral oil and cottonseed oil solutions. Their effects in retarding the formation of peroxides

TABLE II

Effect of Various Concentrations of Phospholipid and Hydroquinone, Used in Combination, on Carotene Stability in Refined Cottonseed Oil. Samples Stored at 75° C.

Concentration of antioxidants		Time for 20% destruction of carotene
Hydroquinone	Phospholipid	
<i>per cent</i>	<i>per cent</i>	<i>hours</i>
1.0	5.0	148
1.0	2.5	140
1.0	0.5	136
1.0	0.05	120
0.5	5.0	120
0.5	2.5	122
0.5	0.5	112
0.5	0.05	105
0.05	5.0	96
0.05	2.5	90
0.05	0.5	85
0.05	0.05	78
0.01	5.0	75
0.01	2.5	72
0.01	0.5	57
0.01	0.05	56

in the refined cottonseed oil were also determined, and similar studies were made on cottonseed oil after the addition of 1.2 mg. of carotene per gram of oil.

In the refined cottonseed oil the formation of peroxides occurred concurrently with the destruction of carotene and was greatly accelerated as the carotene disappeared. In mineral oil solutions, no peroxides were found until after the carotene had become completely decolorized.

Although alpha-tocopherol was very effective in stabilizing carotene when added to mineral oil solutions neither the naturally present nor added tocopherol was effective in a more unstable solvent such as refined cottonseed oil. To make the tocopherol effective in such a solvent it is necessary first to stabilize the solvent. When the combination of phospholipid and hydroquinone was added to the refined

cottonseed oil, the oil was stabilized and a very marked increase in carotene stability was obtained.

While the combinations AH, APH, TAH and TAPH did not stabilize the carotene during the early part of the storage tests, they did become effective after about 20 per cent of the carotene had been destroyed and were as effective as PH and TPH when measured at the 50 per cent point. The reason for this ineffectiveness during the preliminary period of storage is not apparent.

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#### LITERATURE CITED

- (1) A.O.A.C.—*Jour. A.O.A.C.*, **24**, 80 (1941).
- (2) Baumann, C. A., and Steenbock, H., *J. Biol. Chem.*, **101**, 561 (1933).
- (3) Bickoff, E., and Williams, K. T., *Ind. Eng. Chem., Anal. Ed.*, **15**, 266 (1943).
- (4) Golumbic, C., and Mattill, H. A., *J. Am. Chem. Soc.*, **63**, 1279 (1941).
- (5) Golumbic, C., *Oil and Soap*, **20**, 105 (1943).
- (6) Holmes, H., Corbet, R., and Hartzler, E., *Ind. Eng. Chem.*, **28**, 133 (1936).
- (7) Morgal, P. W., Byers, L. W., and Miller, E. J., *Ind. Eng. Chem.*, **35**, 794 (1943).
- (8) Olcott, H. S., *Science*, in press.
- (9) Olcott, H. S., and Mattill, H. A., *J. Am. Chem. Soc.*, **58**, 2204 (1936).
- (10) Olcott, H. S., and Mattill, H. A., *J. Am. Chem. Soc.*, **58**, 1627 (1936).
- (11) Quackenbush, F. W., Cox, R. P., and Steenbock, H., *J. Biol. Chem.*, **145**, 169 (1942).
- (12) Riemenschneider, R. W., Turer, J., Wells, P. A., and Ault, W. C., *Oil and Soap*, **21**, 47 (1944).
- (13) Swift, C. E., Rose, W. G., and Jamieson, G. S., *Oil and Soap*, **19**, 176 (1942).
- (14) Wheeler, D. H., *Oil and Soap*, **9**, 89 (1932).

## Rosin—Use in Salt Water Soap

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The problem of washing on shipboard at sea has probably existed since man built and sailed his first seagoing boat. In the days of sailing ships, it appears that cooking and eating utensils were washed by scrubbing with a mixture of sand and wood ashes. Soap for toilet, bath or laundry use seems to have had little demand. In latter years any available soap seems to have been used until someone hit upon the idea of a salt water soap made from coconut oil.

In this country considerable work has been done on coconut oil soap for this purpose until the product covered by Federal Specification PS611 containing about 3% of salt, 3% of soda ash, 39% coconut oil soap, and 55% water was developed. Few people, it seems, have tried to improve upon it. Pech (1, 2) patented a salt water soap composed of coconut oil 100 parts, caustic soda 4.75 parts, sodium silicate 5 parts, caustic potash 6 parts, and 100 parts of a 4% solution of potassium chlorate. The silicate probably helped the soap but whether or not the chlorate had any value is problematical. Brunet (3) suggested that for washing in sea water a soap containing much glycerol and alkali would remove "epithelial debris" and

grease. Miyoke, Kimoshita and Tomeoka (4) prepared a salt water soap by boiling 2 parts of red algae in 50 parts of water and filtering. To the filtrate, mixed with 18-19 parts of caustic soda and 6-8 parts of soda ash, add 100 parts of palm oil while hot. Then add 1.5-2.5 parts of ammonium chloride and 5 parts of ammonium sulfate. So far as we could discover, nothing was said about how the extract of red algae and the ammonium salts improved the cold process palm oil soap.

The problem of washing in sea water is, of course, that of getting sufficient soap into solution to do the job. In the presence of 25,000 p.p.m. of salt and 12,000 to 13,000 p.p.m. of calcium and magnesium salts, a film of insoluble soap is formed on the surface of an ordinary soap which retards solution to such an extent that the soap is considered insoluble. If the insoluble film is removed as fast as it forms by some kind of abrasion sufficient soap will be dissolved to react with the hardness and saturate the salt solution with soap. One of the physical chemists working in the field of detergents is of the opinion that the film of insoluble soap acts as a dialyzing membrane,