A Study of the Antioxidant Effectiveness of Several Compounds on Vegetable Fats and Oils*

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A great majority of antioxidant studies in the past have been made with animal fats as substrates. In general, the hydrogenation of vegetable oils has stabilized them sufficiently to satisfy ordinary demands. Under the stress of present world circumstances it has become desirable to stabilize vegetable fats to keeping times much greater than was ever before felt necessary. The present communication is a report on the antioxidant effects on vegetable oils and fats of a group of compounds, most of which have been studied in the past with reference to animal fats.

Experimental

Keeping times were measured by the accelerated active oxygen method (Swift stability test) (1,2). In brief, the method measures the length of time required for an air-saturated sample of oil or fat held at 110 ± 0.3° C. to attain a specified peroxide value.

TABLE I

Antioxidant Indices

Concentration of test material added to substrate	Cottonseed oil (9-13)*	Substrates Hydrogenated shortenings†			
		No. S (85-89)*	No. C (67.5)*	No. R (48)*	No. AA (23)*
0.02% NDGA	1.1	....	....	1.4	....
0.05% NDGA	2.1	1.9	2.2	2.1	2.1
0.05% H_3PO_4	....	....	....	....	2.8
0.05% Ascorbyl palmitate	2.1	1.4	1.3	....	1.7
0.03% Ascorbic acid	1.9	....	....	....	....
0.06% Ascorbic acid	2.5	....	....	....	....
0.05% Gallic acid	6.9	3.3	....	....	....
0.05% Gum Guaiac	1.0	....	....	....	....
0.05% Vanillin	0.8	....	....	....	....
0.05% Alloxan	1.4	....	....	....	....
0.05% Glutathione	1.3	....	....	....	....
0.05% Creatine	0.9	....	....	....	....
0.05% dl-methionine	1.5	....	....	....	....
0.05% d-arginine	1.1	....	....	....	....
0.05% l-histidine	0.9	....	....	....	....
0.05% l-cystine	1.1	....	....	....	....
0.05% d-lysine	1.1	....	....	....	....
0.05% VioBin antioxidant	....	1.2	....	....	....
0.05% VioBin No. 5	....	0.9	....	1.6	....
0.05% Siam Benzoin	....	1.1	....	....	....
0.05% Sumatra benzoin	....	1.2	....	....	....
0.05% Syrupy molasses	....	1.1	....	....	....
0.05% Octyl thiodipropionate	....	....	1.4	....	1.4

*Range of induction period on control sample without added antioxidant.
†With lard + 0.05% NDGA an antioxidant index of 20 was obtained.

The standard three-tube method was employed. The change in color produced by natural pigments in vegetable oils was used as the indicator of rancidity in the first tube. This color change has previously been observed by Wheeler (3) and Freyer (4). It was found that just preceding a rapid rise in the rate of peroxide formation in a vegetable oil, a deep yellow to orange color developed. The color completely disappeared immediately following the rapid increase in the rate of peroxidation which characterizes the end of the induction period. By watching this color change, it was possible to know when the first tube had just passed the end of the induction period.

The peroxide values (P. V.) were determined by a modified Wheeler method (3) and were calculated as milliequivalents of $Na_2S_2O_3$ required by one kilogram of sample. The induction period of vegetable oils and fats was measured as the number of hours of aeration necessary to raise the P. V. to 120. In the case of lard, P. V. 20 was used as the critical point.

The effect of various antioxidants on the induction periods of several oil samples have been assembled in

TABLE II

Antioxidant Indices

Concentration of test material added to substrate	Cottonseed oil (9-13)*	Substrates	
		Hydrogenated shortening No. AA (23)*	Lard (2.5)*
0.05% NDGA + 0.01% H_3PO_4	....	4.7	....
0.05% NDGA + 0.05% H_3PO_4	....	6.4	24.8
0.05% NDGA + 0.1% H_3PO_4	....	>>6.4	....
0.05% NDGA + 0.05% citric acid		5.0	....
0.03% gallic acid + 0.03% ascorbic acid	4.5	....	....
0.03% gallic acid + 0.01% H_3PO_4	2.4	....	....
0.06% gallic acid + 0.06% ascorbic acid	6.5	....	....

*Range of induction period on control sample without added antioxidant.

Tables I and II. These keeping times were calculated to correspond with values which would have been obtained at 97.7° C. in the original unaccelerated Swift stability test (1). The actual time of aeration at 110° C. is multiplied by 2.5 (2). The data in Tables I and II are expressed in terms of the antioxidant index, the quotient of the keeping times with and without antioxidant. The index is a measure of the relative effectiveness as an antioxidant. It must be kept in mind that the vegetable oils and fats contained natural inhibitors, and thus the effects observed are the results of combinations of antioxidants.

Discussion of Results

In the determination of the antioxidant indices of the various compounds, several different substrates were used. They included samples of refined cottonseed oil, several hydrogenated shortenings, and a sample of home-rendered lard. On the basis of the different substrates there was one observation of note. Regardless of the vegetable substrate used, one of the antioxidants, NDGA† in 0.05% concentration, always appeared to about double the keeping time. Such a phenomenon was not evident for any of the other antioxidants studied.

The compounds examined most closely were NDGA, ascorbyl palmitate‡ and gallic acid. With lard, the antioxidant index of NDGA was quite high, although not so high as has been reported by Lundberg *et al.*

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† NDGA—nordihydroguaiaretic acid (6).
‡ 6-palmityl ascorbic acid, prepared by method of Swern, *et al.* (5).

(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.

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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 suggested 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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