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A Comparative Evaluation of Several Antioxidants in Edible Fats[1]

R. N. MOORE[2] and W. G. BICKFORD, Southern Regional Research Laboratory,[3] New Orleans, Louisiana

DURING recent years a number of compounds have become available for use in stabilizing fats and oils against oxidative deterioration. The majority of these antioxidants are phenols of widely different chemical structures varying from relatively simple compounds like sesamol to the more complex phenols such as the conidendrols.

Considerable information is available relative to the antioxidant activity of some of these compounds while others appear to have been relatively neglected. Even where considerable data are available, they may refer to a single substrate and to synergistic activity of the compound rather than to its antioxidant activity. It is often difficult, if not impossible, to obtain from the literature comparative data with respect to the effectiveness of various antioxidants for a particular substrate. This results from the fact that substrates employed by different workers are generally not identical. Frequently inadequate information is presented regarding the substrate, especially with respect to its freshness and initial peroxide content. Comparison of available data also suffers from differences in methods employed for calculating and expressing the activity of the antioxidant tested.

Although cottonseed oil is recognized as having good stability toward oxidative rancidity, much effort has been expended in searching for methods of further improving this property. Many of the efforts in this direction have suffered from the inadequacies mentioned above. The present investigation has been conducted so as to obviate as far as possible the aforementioned difficulties in intercomparing antioxidants. To this end it appeared expedient to a) use fresh substrates of known history, b) intercompare a number of new as well as older substances known to possess antioxidant activity, c) establish curves for the accumulated peroxide content of a given substrate for three concentrations of inhibitor rather than to make a minimum number of peroxide value determinations and terminate the test at an arbitrarily pre-established peroxide value.

The three substrates used were a) finished edible cottonseed oil, b) cottonseed oil hydrogenated to shortening consistency, and c) prime steam lard, the latter being relatively low in natural antioxidants. Thirteen antioxidants, 12 of which are representative of various phenolic types, were tested with respect to their antioxidant activities in each of the substrates and at three concentrations.

Experimental

Substrates. Prime grade cottonseed was cleaned, delinted, moistened, hulled, and the separated meats flaked and extracted with commercial hexane at 120° F. in a continuous solvent extraction pilot plant. The miscella was distilled and the oil stripped of solvent at temperatures which did not exceed 180° F. The crude oil was settled for two weeks prior to refining. A 30-pound batch of the crude oil (f.f.a. 1.05%) was alkali-refined in a stainless steel kettle with 14° Bé. caustic soda, using an 0.8% excess of dry sodium hydroxide on the basis of weight of oil. The cold oil and alkali were stirred rapidly for 15 minutes, heated to 65° C., and stirred slowly for an additional 12 minutes. After settling over-night, the oil was decanted, washed, filtered, and bleached by stirring for 15 minutes with B.C.[4] clay at 110° C. The refined and bleached oil had a color of 10 yellow and 1.1 red Lovibond units. Steam deodorization in an all-glass laboratory apparatus (2) yielded a bland oil with zero peroxide value.

A quantity of the refined and bleached cottonseed oil (iodine value 100.4) was selectively hydrogenated to shortening consistency in a laboratory hydrogenator (3) at 190° C. and 15 p.s.i. pressure in the presence of 0.1% of electrolytically-precipitated, dry-reduced, nickel catalyst (1). The hydrogenated oil (iodine value 61.4) after filtration and deodorization had a zero peroxide value.

The third substrate was a fresh, commercial prime steam-rendered lard, free of added antioxidants and peroxides when received.[5]

Each of the substrates was filled into 4-ounce bottles previously purged with carbon dioxide. The samples were then stored at 0° C. until used. When the bottle was opened to remove a sample, the remaining portion was discarded.

During the entire storage period, during which the tests were conducted, the peroxide value of the cottonseed oil increased to a value of 2 and the lard to a value of 3 milliequivalents, while that of the hydrogenated oil remained unchanged.

Antioxidants. The compounds tested for antioxidant activity were butylated hydroxyanisole, α-conidendrol,

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[2] Present address, Naval Stores Station, Bureau of Agricultural and Industrial Chemistry, Olustee, Fla.

[3] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

[4] The mention of this product does not imply that it is endorsed or recommended by the Department of Agriculture over others having similar properties but is mentioned as a part of the exact experimental conditions employed.

[5] The authors wish to express their appreciation to H. T. Spannuth, Wilson and Company, Chicago, Ill., for his cooperation in supplying the carefully processed lard used in this investigation.

TABLE I

Comparison of Antioxidant Activities of Various Inhibitors in Cottonseed Oil and in Hydrogenated Cottonseed Oil[a]

Antioxidant	Cottonseed oil[b]			Hydrogenated cottonseed oil[b, c]		
	0.01%	0.05%	0.10%	0.01%	0.05%	0.10%
Control[d]	9.1	9.1	9.1	121	121	121
α-Tocopherol	7.6	7.1	6.3	107	102	92
γ-Tocopherol	9.0	8.3	6.8	135	161	177
Lecithin	10.6	11.3	12.1	172	186	181
α-Conidendrol	10.1	13.0	20.9	153	270	412
β-Conidendrol	11.4	17.5	25.6	174	347	453
Norconidendrin	14.1	22.1	32.5	152	244	388
Nordihydroguaiaretic acid	10.0	18.7	25.8	120	258	393
Gum guaiac	6.6	8.5	9.3	100	124	152
Hydroquinone	9.3	35.0	49.0	128	273	392
Sesamol	8.8	15.5	17.2	98	156	194
Propyl gallate	15.8	54.9	79.5	172	495	614
Butylated hydroxyanisole	6.6	7.3	7.3	108	158	172
Di-*tert*-butyl-*p*-cresol	9.4	14.6	18.0	118	172	184

[a] The relative antioxidant activity is expressed as the number of hours required by the stabilized substrate to acquire a peroxide content of 100 milliequivalents per kilogram during aeration at 97.7° C. with an air flow of 2.33 ml./sec.
[b] Substrates and percentages of antioxidants incorporated therein.
[c] Iodine value of hydrogenated oil was 61.4 as determined by the Wijs method using a 30-minute reaction time.
[d] Substrate without added antioxidant.

TABLE II

Comparison of the Antioxidant Activities of Various Inhibitors in Lard

Antioxidant[a]	AOM hours[b]					
	At 20 Me./kg.			At 100 Me./kg.		
	0.01%	0.05%	0.10%	0.01%	0.05%	0.10%
Control[c]	4.1	4.1	4.1	5.0	5.0	5.0
α-Tocopherol	17.0	11.3	5.4	18.5	25.5	26.0
γ-Tocopherol	19.3	18.0	11.3	30.0	49.5	47.5
Lecithin	4.8	5.9	7.0	5.5	6.0	8.0
α-Conidendrol	25.5	43.2	43.7	33.1	99.0	136.0
β-Conidendrol	32.5	53.0	51.5	44.0	110.0	146.0
Norconidendrin	25.8	50.0	48.8	31.0	103.0	141.0
Nordihydroguaiaretic	50.0	42.0	35.0	62.5	116.0	151.0
Gum guaiac	3.4	9.0	12.3	4.5	16.0	23.8
Hydroquinone	65.0	122.0	148.0	70.0	131.0	145.0
Sesamol	30.8	65.0	74.5	38.5	83.0	103.0
Propyl gallate	43.8	90.0	88.0	47.5	160.0	196.0
Butylated hydroxyanisole	19.3	20.4	21.4	31.0	56.0	68.0
Di-*tert*-butyl-*p*-cresol	22.7	50.0	68.0	26.5	50.0	79.0

[a] Antioxidant expressed as percentage incorporated in substrate.
[b] Number of hours required by the substrate to accumulate stated milliequivalents of peroxide per kilogram during aeration at 97.7°C. with an air flow of 2.33 ml./sec.
[c] Contains no added antioxidant.

β-conidendrol, di-*tert*-butyl-*p*-cresol, gum guaiac, hydroquinone, lecithin, norconidendrin, nordihydroguaiaretic acid, propyl gallate, sesamol, α-tocopherol, and γ-tocopherol. Norconidendrin (6) and sesamol (4) were synthesized in this laboratory. All of the other products were of commercial origin.

Method. Each of the above-mentioned compounds was incorporated in each of the substrates in concentrations of 0.01, 0.05, and 0.10% with the aid of 1-3 ml. purified absolute ethanol. An equivalent amount of ethanol was added to the control sample. Portions of 20 grams each were placed in test tubes previously cleaned with a detergent (7) and aerated with purified air at the rate of 2.33 ml./sec. (5, 8) at 97.7° C.

At regular intervals a 1-g. sample of fat was removed and the peroxide value determined as follows: The sample was dissolved in 25 ml. of acetic acid-chloroform (3:2), swirled for 2 minutes with 0.50 ml. of freshly prepared saturated potassium iodide solution, followed by dilution with 50 ml. distilled water. The liberated iodine was immediately titrated with 0.01 *N* sodium thiosulfate solution. The peroxide value was calculated as milliequivalents per kilogram of substrate (Me./kg.). All stability measurements were made by aeration of duplicate samples of the materials tested. Generally duplicate samples agreed within 5%, although agreement within 10% was considered satisfactory where the "keeping time" was 100 hours or more. In only 3% of all the determinations (7), approximately 4,000, was unsatisfactory agreement in duplicate samples observed.

Discussion of Results

The results of the antioxidant activity tests are given in Tables I and II, and typical peroxide accumulation curves are reproduced in Figures 1-5. For the sake of clarity the majority of points used in plotting the curves have been omitted from the reproduced figures, and for the same reason many of the curves have been omitted.

The stability data reported in Table I are based on the time required for the aerated samples to attain a peroxide value of 100 Me./kg. while those for lard in Table II are calculated on a basis of 20 Me./kg. as well as 100 Me./kg.

In general, the data are self-explanatory, but a few points require comment and generalization. These may be summarized as follows:

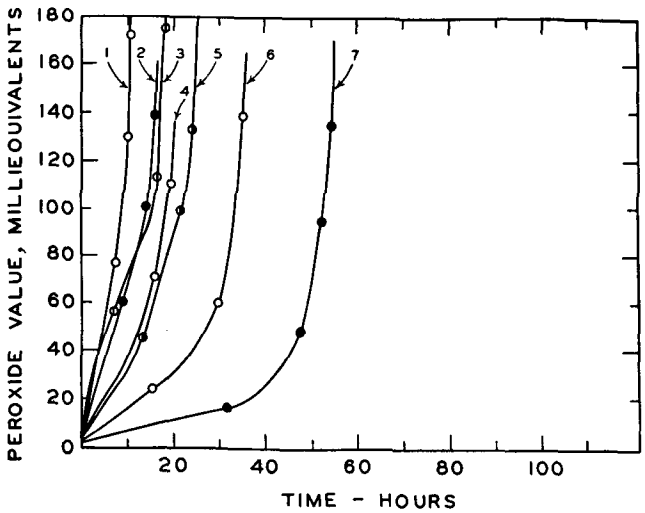

FIG. 1. Cottonseed oil containing 0.05% antioxidant. (1) control, (2) di-*tert*-butyl-*p*-cresol, (3) sesamol, (4) nordihydroguaiaretic acid, (5) norconidendrin, (6) hydroquinone, and (7) propyl gallate.

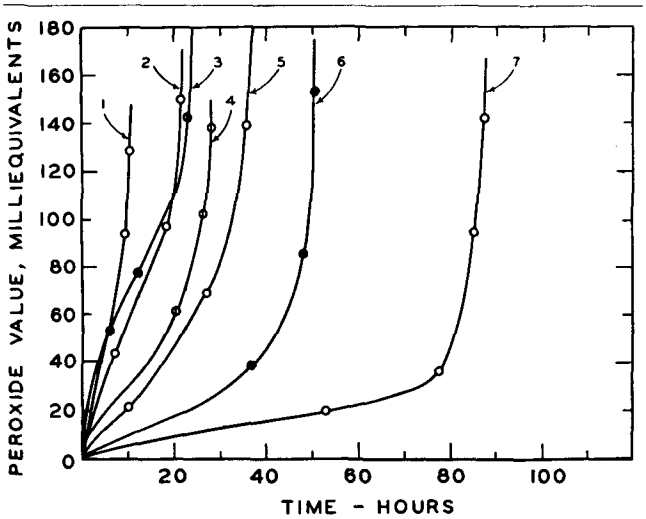

FIG. 2. Cottonseed oil containing 0.10% antioxidant. (1) control, (2) di-*tert*-butyl-*p*-cresol, (3) sesamol, (4) nordihydroguaiaretic acid, (5) norconidendrin, (6) hydroquinone, and (7) propyl gallate.

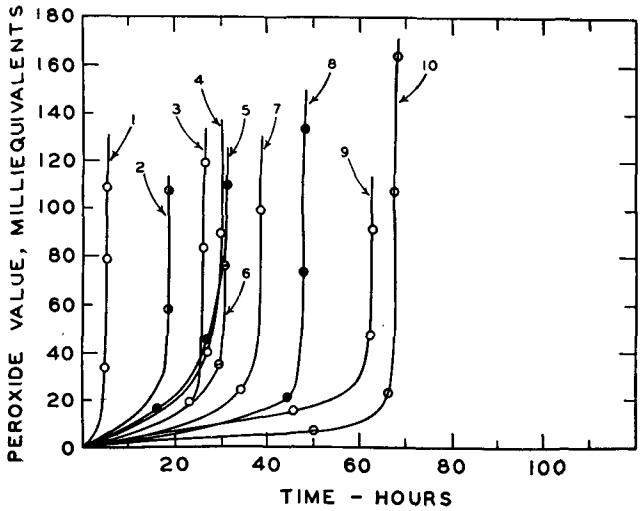

FIG. 3. Lard containing 0.01% antioxidant. (1) control, (2) α-tocopherol, (3) di-*tert*-butyl-*p*-cresol, (4) γ-tocopherol, (5) butylated hydroxyanisole, (6) norconidendrin, (7) sesamol, (8) propyl gallate, (9) nordihydroguaiaretic acid, and (10) hydroquinone.

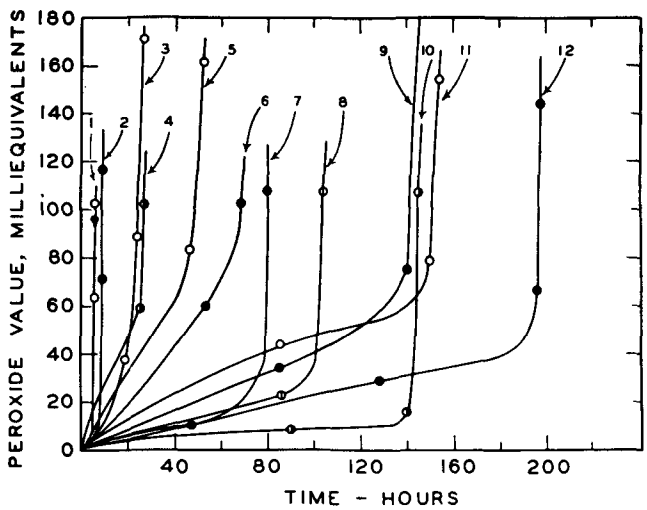

FIG. 5. Lard containing 0.10% antioxidant. (1) control, (2) lecithin, (3) gum guaiac, (4) α-tocopherol, (5) γ-tocopherol, (6) butylated hydroxyanisole, (7) di-*tert*-butyl-*p*-cresol, (8) sesamol, (9) norconidendrin, (10) hydroquinone, (11) nordihydroguaiaretic acid, and (12) propyl gallate.

a) Except for lecithin, all of the antioxidants tested proved to be more effective in stabilizing lard than in stabilizing cottonseed oils.

b) The relative effectiveness of an antioxidant compared to that of any other antioxidant in a given substrate depends on the concentration. For example, hydroquinone is more effective than propyl gallate at .01% concentration in lard whereas the reverse is true at .05 and .10% concentrations.

c) Propyl gallate was found to be the most effective antioxidant for hydrogenated and unhydrogenated cottonseed oils. This was also true for lard for the two highest concentrations employed.

d) Nordihydroguaiaretic acid, hydroquinone, the conidendrols, and norconidendrin are intermediate in antioxidant activity in the cottonseed oils. None of the other compounds in the concentrations employed more than doubled the stability of these oils.

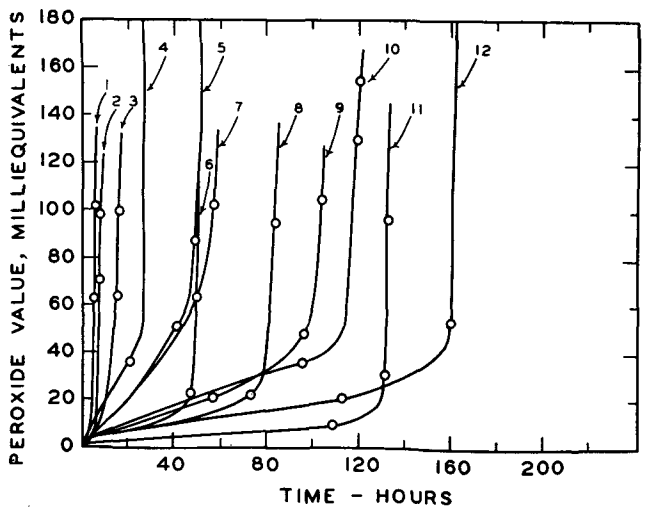

FIG. 4. Lard containing 0.05% antioxidant. (1) control, (2) lecithin, (3) gum guaiac, (4) α-tocopherol, (5) γ-tocopherol, (6) di-*tert*-butyl-*p*-cresol, (7) butylated hydroxyanisole, (8) sesamol, (9) norconidendrin, (10) nordihydroguaiaretic acid, (11) hydroquinone, and (12) propyl gallate.

e) In contrast to their behavior in lard, low concentrations of the inhibitors are without significant effect in the cottonseed oils.

f) The resin acid-type inhibitors had a greater stabilizing action in hydrogenated than in unhydrogenated cottonseed oil. This behavior is in contrast to that of less complex phenols which are more active in the unhydrogenated oils.

g) Mattil *et al.* (9) reported that nordihydroguaiaretic acid in 0.05% concentration approximately doubled the keeping time of vegetable oils. The results of similar tests shown in Table I further confirm the observations of these workers.

h) Inspection of the peroxide accumulation curves for the lard shows that although a marked increase in the rate of peroxide accumulation occurs in the control sample in the region of 20 Me./kg., the corresponding point of increase in antioxidant-treated sample is displaced toward a higher peroxide value. In either case the rate of peroxide accumulation approaches infinity above the knee of the curve. It is generally true that organoleptic rancidity is unmistakably detectable in the aerated sample at a time coincident with a rapid increase in the rate of peroxide accumulation indicated by the knee of the peroxide-time curve, and there is some evidence to show that the antioxidant is also depleted at the same time (4, 10). These observations suggest that the determination of antioxidant efficiency in lard could be based on a peroxide value of 100 Me./kg. as in the case of vegetable oils. This procedure yields a more realistic evaluation of the antioxidant activity of any given compound. For example, inspection of curve No. 12 of Figure 5 for lard containing 0.10% propyl gallate shows that 90 hours of aeration produced a peroxide value of 20 but that aeration of 190 hours and a peroxide value of 45 was required before the knee developed in the curve. It may also be noted from the same curve that the peroxide value increased from 45 to 100 in a very short time. While the evaluation of antioxidant efficiency would perhaps be more rational if it were based on the time-peroxide value relationship existing at the knee of the curve, it is obvious that use of a peroxide

value of 100 introduces only a negligible error. Reference to Figures 3, 4, and 5 and corresponding data given in the tables clearly illustrate this point.

Summary

Thirteen compounds have been intercompared with respect to their antioxidant activity in concentrations of 0.01, 0.05, and 0.10% in edible cottonseed oil, the same oil hydrogenated to shortening consistency, and in lard which is essentially free of naturally occurring antioxidants. None of the compounds exhibited significant antioxidant activity in the cottonseed oils, when used in a concentration of 0.01%, but they were effective in stabilizing lard under these conditions. Propyl gallate was the most effective of the compounds tested for the vegetable fats.

Examination of the data for lard indicates that the comparison of antioxidants in this and other substrates essentially devoid of natural inhibitors may yield more realistic results when compared at a peroxide level of 100 milliequivalents per kilogram of substrate.

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Buckwheat Leaf Meal Fat. II. Composition of the Fatty Acids

CHARLES F. KREWSON, Eastern Regional Research Laboratory,[1] Philadelphia, Pennsylvania

A PREVIOUS paper (5) describes the preparation of buckwheat leaf meal fat, reports its physical and chemical characteristics, and gives an account of the identification of the constituents in the unsaponifiable matter and the water-soluble fraction of the saponified fat. This manuscript describes the characterization of the chief constituents in the water-insoluble fraction of the saponified fat.

Purification of the Water-Insoluble Fatty Acids. According to Official Methods (1), the value for insoluble acids includes the unsaponifiable matter. The quantity of unsaponifiable matter is inconsequential in most fats and oils that contain these constituents in low concentration, but since buckwheat fat contains a high percentage (14.9%) of this material, the value of 64.9% previously reported (5) for the insoluble acids did not include the unsaponifiable matter.

The insoluble acids (free of unsaponifiable matter) contain a quantity of material not of fatty acid character. Exhaustive extraction of a 221.8-g. sample of the insoluble acids with petroleum ether (boiling range 63-70°C.) gave 158.6 g. (71.5%) of soluble material and 63.2 g. (28.5%) of insoluble material. On the basis of the original fat the petroleum-ether soluble material (fatty acids) was 46.6%, and the insoluble material was 18.5%.

To free the purified fatty acids of a small amount of coloring matter, an 89.4-g. sample of the soluble material was dissolved in petroleum ether and treated with 40.0 g. of Norit. To determine whether appreciable quantities of individual acids were preferentially adsorbed by Norit, untreated and Norit-treated samples were submitted to spectrophotometric analyses (2, 3) for polyunsaturated acids and to various chemical analyses for analytical characteristics. Table I shows the results of these analyses.

TABLE I

Spectrophotometric and Analytical Characteristics of Purified Mixed Fatty Acids of Buckwheat Leaf Meal Fat

	Pigment-contaminated fatty acids	Pigment-free fatty acids
Peroxide value (milliequivalents/kilo)	24.2	25.9
Iodine value (Wijs)	154.8	163.3
Neutralization equivalent	296.0	285.7
Hydroxyl value (%)		0.1
Saturated acid content (%)[a]	21.8	22.0
Saturated acids by difference (%)	17.2	19.7
Neutralization equivalent, saturated acids from Bertram analysis[b]	295.0	282.2
Iodine value, saturated acids from Bertram analysis		0.4
Oleic acid, calculated from iodine value and spectrophotometric analyses (%)	28.2	18.7
Linoleic acid (%)	18.2	20.9
Linolenic acid (%)	32.5	37.5
Preformed conjugated dienoic acids (%)	3.5	3.0
Ratio linolenic acid/linoleic acid	1.797	1.794

[a] Bertram method as modified by G. S. Jamieson, "Vegetable Oils and Fats," A.C.S. Monograph No. 58, 2nd Edition, 414-415 (1943).
[b] A single determination of neutralization equivalent was made on the saturated acids obtained from the Bertram analysis.

Both samples showed some spectroscopic evidence of oxidation by their appreciable diene absorption before isomerism and their tetraenoic absorption on heating in the absence of alkali (10). They did not appear to have reached a high state of oxidation however, and the differences in the degrees of oxidation of the samples were minor. The peroxide values of the samples substantiated the spectrophotometric findings. The linolenic/linoleic acid ratio alone indicates that Norit exerts little fractionating effect on the fatty acids. Calculations made from spectrophotometric analyses and iodine values suggest however that oleic acid may be preferentially absorbed by Norit.

Purification of the fatty acids may remove some substance which offers interference in the linolenic acid determination. Any analytical error in this determination would be multiplied in the calculation of oleic acid. In view of the uncertainty concerning

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.