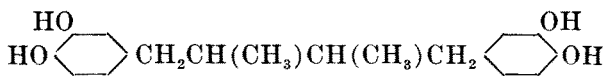
The Antioxidant Properties of Nordihydroguaiaretic Acid*

W. O. LUNDBERG, H. O. HALVORSON and G. O. BURR
Division of Physiological Chemistry
University of Minnesota, Minneapolis

Numerous phenolic substances have the ability at low concentrations to markedly inhibit the autoxidation of fats. In general, the most effective phenols are those which have some type of oxygen linkage in the ortho or para positions, or both, to the hydroxyl group. The simplest and best known antioxidant of this type is hydroquinone. Other examples are the tocopherols which have been extensively investigated (1-7) because they appear to be the principal antioxidants occurring naturally in many vegetable and animal fats. They have the advantages of being less toxic and more fat-soluble than hydroquinone. Grettie has reported antioxygenic characteristics of gum guaiac (8) and this substance is now used in some commercial lards. The active principle in gum guaiac is presumably guaiaretic acid.

Following favorable preliminary results, an investigation was made of the antioxidant properties of nordihydroguaiaretic acid (hereinafter abbreviated to NDGA):



It was synthesized in 1918 from hydroguaiaretic acid ether and its structure established (8-a). In the course of investigations on plant constituents being conducted cooperatively by the U. S. Department of Agriculture and the University of Minnesota it was found that NDGA occurs in a common desert plant, *Larrea divaricata* (9), one of several plants known as creosote bush. The dried leaves and stems of random samples of the plant analyzed in this laboratory contained up to 7 per cent of NDGA. The pure compound may be prepared by recrystallization of the crude extract.

General Observations

Pure NDGA is a white crystalline solid (m.p. 184-5°C.) very slightly soluble in water and dilute acids, moderately soluble in hot benzene and xylene, and very soluble in diethyl ether, alcohol, and glacial acetic acid. It dissolves appreciably in hot fats (e.g. lard at 125-150°C.) and does not readily precipitate when the fats are cooled. It may be brought into solution in liquid fats at room temperature using alcohol as a vehicle and subsequently removing the alcohol by evaporation.

In obtaining the data presented here, lards were the principal substrates used. At the low concentrations of NDGA used for antioxidant purposes, no appreciable color developed when the lards were heated to their smoke points. Concentrations up to 0.1 per cent imparted no taste or odor that could be detected by the average person, and the shortening properties were not altered. Preliminary toxicity studies on rats, mice and guinea pigs indicate that concentrations considerably higher than those necessary for the stabilization of lards are physiologically harmless.

Antioxidant Properties

Two accelerated tests were used in measuring stabilities, the Swift stability test at 98.6°C. (10) and an oven test at 63 ± 0.5°C. In the latter, samples weighing 50 grams were kept in 100 ml. beakers, and the organoleptic testing was supplemented by peroxide determinations made essentially by the Wheeler iodometric method (11). A few measurements were also conducted at room temperatures.

Figure 1 is a comparison of the peroxide accumulation curves at 63°C. of a sample of standard run steam rendered lard containing 0.1 per cent of NDGA having an estimated purity of about 95 per cent and a control sample of the same lard. The protective index is about 48, based on the times at which the odor of oxidative rancidity was first detected in the two samples. It is observed that the end of the induction period, whether it be defined by the point at which the slope of the curve increases sharply or by the point at which rancidity is first detected, occurs at a considerably higher peroxide level when the antioxidant is present. Tocopherols produce the same phenomenon (4), and the effect is probably common to most if not all phenolic inhibitors.

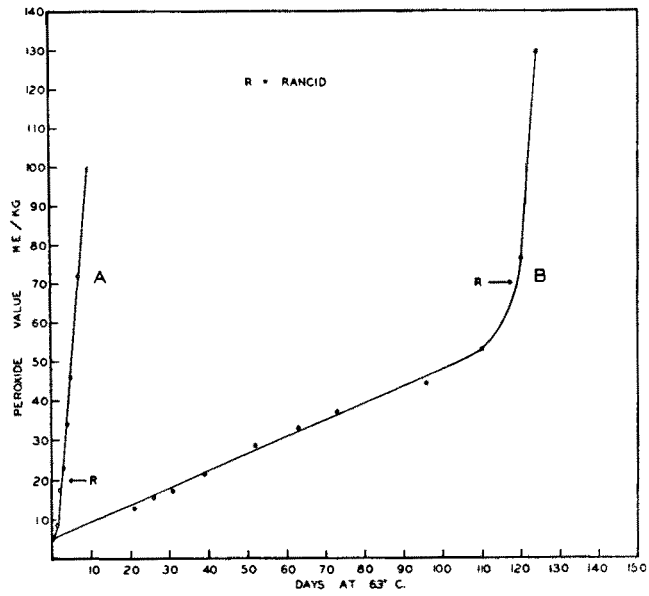


FIG. 1. Oven test at 63°C.
A. Standard run steam rendered lard (control).
B. Lard containing 0.1 per cent NDGA.

The effects of hydroquinone, alpha tocopherol, and NDGA in two lards are compared in Table I. These special lards were prepared from the mesenteric fat

* This work was done under the auspices of the Hormel Research Foundation.

TABLE I

The Swift Stabilities of Lards Containing Hydroquinone, Alpha tocopherol, and NDGA

Substrate	Antioxidant		Swift keeping time	Protective Index
			Hrs.	
Special Lard No. 1	Control		11	....
	Hydroquinone	.01%	84	8
	Hydroquinone	.05%	190	17
	Alpha tocopherol	.01%	33	3
	Alpha tocopherol	.05%	97	9
	NDGA	.01%	210	19
	NDGA	.05%	279	25
Special Lard No. 2	Control		23	....
	Hydroquinone	.02%	170	7
	Hydroquinone	.10%	310	13
	NDGA	.02%	270	12
	NDGA	.10%	370	16
Standard Run Steam Rendered Lard	Control		3	....
	NDGA	.02%	96	32
	NDGA	.10%	168	56

of hogs. Results are given also for two concentrations of NDGA in a standard run steam rendered lard of poor keeping quality; the relative effectiveness of the antioxidant appears to be considerably greater in this substrate, due probably to the absence of any appreciable amount of natural antioxidant. It is shown later (Table III) that when alpha tocopherol and NDGA are both added to a lard, the keeping time of the sample is no greater than when the same concentration of NDGA is added alone. Because the special lards had been carefully rendered and retained 4-10 γ of natural tocopherol per gram of fat (unpublished data), the relative antioxidant effectiveness of the NDGA in the special lards is decreased.

It is well recognized that accelerated tests do not always yield reliable information about the stability of fats under commonly used storage conditions. Samples weighing 50 grams were therefore prepared and set aside in 100 ml. beakers at prevailing room temperatures (70-85°F.) in diffuse daylight. Table II

TABLE II

Effectiveness of NDGA in Lards at Room Temperature in Diffuse Daylight

Sample	Time	Condition
1. Standard run steam rendered lard	6 weeks*	P.V. > 20, rancid
2. Same as (1), plus .05% NDGA	50 weeks	P.V. = 14.4, not rancid
3. Special lard No. 2	22 weeks*	P.V. > 20, rancid
4. Same as (3), plus .05% NDGA	50 weeks	P.V. = 10.2, not rancid

* Elapsed time before rancidity was first detected.

summarizes the incomplete data that were obtained. The peroxide values (P. V.) are given in milli-equivalents per kilogram of lard. In Figure 1 it was shown that under certain conditions the antioxidant permits considerably higher peroxide levels to be reached before the induction period ends. In the presence of diffuse daylight, however, the same effects may not be obtained, and it is therefore impossible to predict how much longer samples 2 and 4 in Table II would remain organoleptically fresh.

The stabilizing effect of NDGA as a function of initial concentration is shown for two lards in Figure 2. It has previously been shown (4, 6) that there are limiting concentrations of tocopherols above which no further increase in stability is achieved. Similar results are indicated in Figure 2, and little appears to be gained by using concentrations of NDGA in lards above .05 per cent.

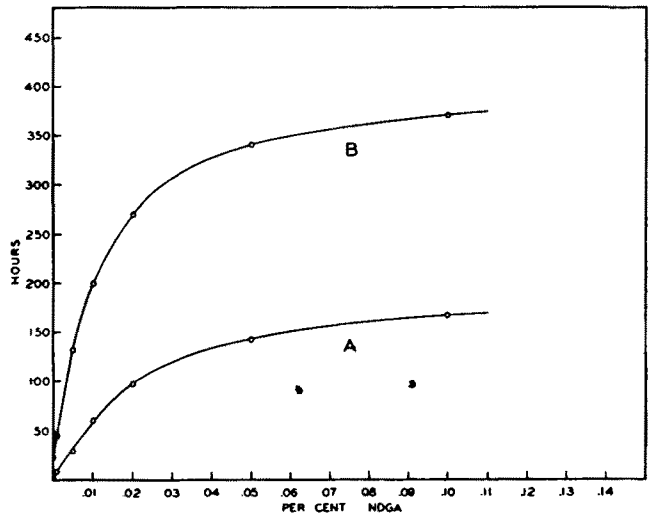


FIG. 2. Stability of lard as function of initial NDGA concentration (Swift stability test).

A. Standard run steam rendered lard.

B. Special lard prepared from mesenteric fat.

Tests were conducted to determine if any of the stabilizing effect of the antioxidant could be carried over into baked products. Pie crusts were prepared using a popular recipe and soda crackers were made by a laboratory method (12). The products were subjected to the Rabak test as described by Bohn and Olson (12) and from time to time samples were taken from the oven and the fats extracted and analyzed for peroxide content, Figures 3 and 4.

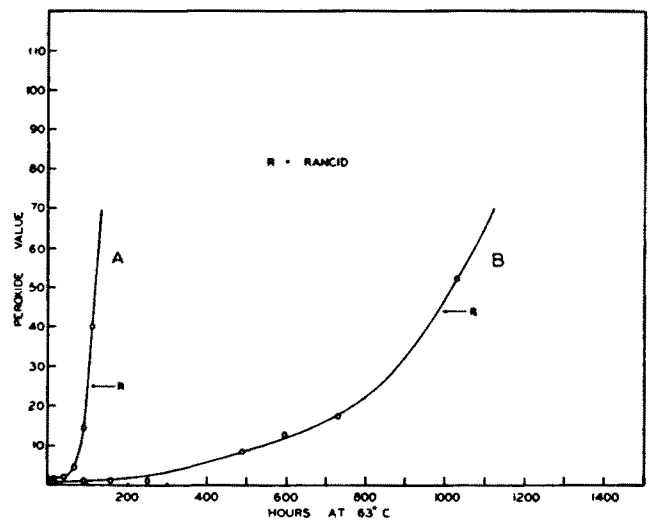


FIG. 3. Rabak test on pie crusts at 63°C.

A. Special lard, Swift stability—23 hours.

B. Same lard containing 0.1 per cent NDGA, Swift stability —370 hours.

The retention of antioxidant effectiveness was greater in the pie crusts than in the soda crackers. Alkaline solutions of NDGA oxidize rapidly when exposed to air; the lesser effectiveness of the NDGA in the soda crackers may therefore be due to the alkalinity imparted by the baking soda included in this product.

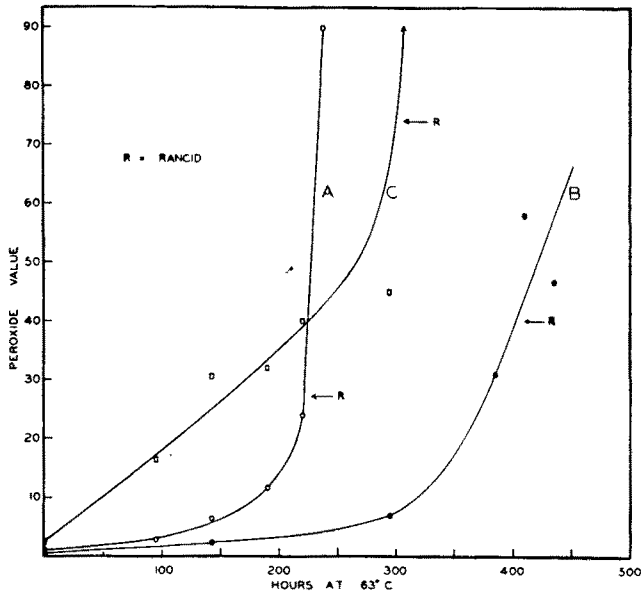


FIG. 4. Rabak test on soda crackers at 63°C.

A. Special lard, Swift stability—23 hours.

B. Lard containing 0.1 per cent NDGA, Swift stability—370 hours.

C. Hydrogenated vegetable oil.

The synergistic effect of various substances on the antioxidant effectiveness of tocopherols and inhibitols has been described (3, 5, 13). An experiment was conducted to determine whether NDGA exerted any synergistic effect on the antioxygenic activity of a wheat germ concentrate containing 40 per cent of mixed tocopherols. The results in Table III show that

TABLE III

The Stability of Lard Containing a Wheat Germ Concentrate (40% Tocopherols) and NDGA

Sample	Swift keeping time
	Hours
1. Standard run steam rendered lard	4
2. Lard + .04% wheat germ concentrate	35
3. Lard + .01% NDGA	76
4. Lard + .04% wheat germ conc. + .01% NDGA	71

the two ingredients in combination exert no greater stabilizing effect on lard than the NDGA alone. Table IV, however, shows that ascorbic acid enhances the

TABLE IV

The Stability of a Lard Containing NDGA and Ascorbic Acid

Sample	Swift keeping time	Increase in keeping time due to antioxidant
	Hours	*Hours*
1. Special lard	20	....
2. Lard + .05% ascorbic acid	68	48
3. Lard + .01% NDGA	206	186
4. Lard + .05% ascorbic acid + .01% NDGA	405	385

stabilizing effectiveness of NDGA. The magnitude of the effectiveness of ascorbic acid alone on the substrate may be attributed to its synergistic effect on the natural antioxidants in the lard.

Summary

A description is given of the antioxidant properties of nordihydroguaiaretic acid. This substance is readily obtained in substantial yields from a common plant *(Larrea divaricata)* and compares favorably with other highly effective inhibitors of the phenolic type.

It is more soluble in fats than hydroquinone but not as soluble as the tocopherols. Within the limits of these experiments, and at optimal concentrations, it appears to have no deleterious effects on the qualities of lards.

Its effectiveness in stabilizing fats is to some extent carried over into baked products.

Ascorbic acid enhances its effectiveness.

Acknowledgments

The authors wish to acknowledge the assistance given by the following members of the staff of the University of Minnesota: Dr. Walter M. Lauer who suggested the trial of NDGA as an antioxidant; Drs. Ole Gisvold and Raymond N. Bieter who were conducting the cooperative experiments with the United States Department of Agriculture and supplied samples of NDGA used in the early experiments; and Dr. R. H. Barnes who made preliminary toxicological studies and gave valuable suggestions. Extensive toxicological studies now being made by Dr. Bieter, Department of Pharmacology, University of Minnesota, will be published later.

Acknowldegments are also due Mr. Jacques Chipault for assistance in the analytical work, Mrs. R. H. Barnes for preparation of the baked products, and Distillation Products, Inc., Rochester, N. Y., for a sample of wheat germ concentrate.

BIBLIOGRAPHY

1. Olcott, H. S. and Emerson, O. H., J. A. C. S. *59*, 1008 (1937).

2. Golumbic, C., J. A. C. S. *63*, 1142 (1941).

3. Golumbic, C. and Mattill, H. A., J. A. C. S. *63*, 1279 (1941).

4. Swift, C. E., Rose, W. G. and Jamieson, G. S., Oil and Soap *19*, 176 (1942).

5. Golumbic, C., Oil and Soap *19*, 181 (1942).

6. Golumbic, C., Oil and Soap *20*, 105 (1943).

7. Barnes, R. H., Lundberg, W. O., Hanson, H. T. and Burr, Geo. O., J. Biol. Chem. *149*, 313 (1943).

8. Grettie, D. P., Oil and Soap *10*, 127 (1933).

8a. Schroeter, G., Lichtenstadt, L. and Irineu, D., Ber. *51*, 1587 (1918).

9. Waller, C. W., Ph.D. Thesis, University of Minnesota (1942).

10. King, A. E., Roschen, H. L. and Irwin, W. H., Oil and Soap *10*, 105 (1933).

11. Wheeler, D. H., Oil and Soap *9*, 89 (1932).

12. Bohn, R. M. and Olson, R. S., Oil and Soap *11*, 210 (1934).

13. Golumbic, C. and Mattill, H. A., Oil and Soap *19*, 144 (1942).