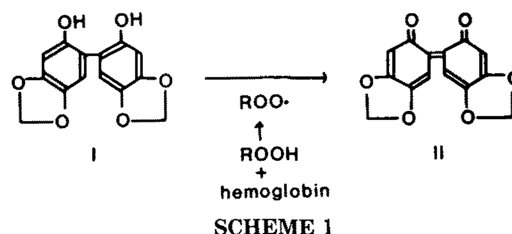
A Sensitive Test to Evaluate Antioxidants in Oils and Fatty Esters

Kiyomi Kikugawa*, Takami Nakahara and **Masami Tanaka**
Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan

Efficacy of common lipid antioxidants was evaluated by use of sesamol dimer and hemoglobin (SD method), and the method was compared with traditional peroxide value (POV) method. There were good correlations between the increases in SD and POV values, with different substrate oils and with the different antioxidants. While the POV method requires 500 mg of the substrate oil, the SD method requires less than 10 mg of the oil. The SD method is useful to estimate the potency of antioxidants with a small amount of oils and fatty esters.

Evaluation of efficacy of lipid antioxidants is based on the inhibition of accumulation of lipid hydroperoxides in oils and fats, primary products of lipid autoxidation (1). Measurement of peroxide value (POV) of the substrate oils containing the antioxidants is most widely used for this purpose (2,3). This method, however, suffers from limited sensitivity and requires a large amount of oils and fats for accurate determination of lipid hydroperoxides. The thiobarbituric acid test is also useful, but it is not specific for lipid hydroperoxides (4,5).

Previously we developed a specific determination of lipid hydroperoxides by use of sesamol dimer (I) and hemoglobin (SD method) (6,7). The principle of the assay is based on the formation of violet-colored quinone (II) from I by the active species produced in the reaction of lipid hydroperoxides and hemoglobin (Scheme 1). In this paper, we applied the SD method to evaluate the efficacy of common antioxidants of oils and fats and found that it is useful with a small amount of oils and fatty ester samples.



SCHEME 1

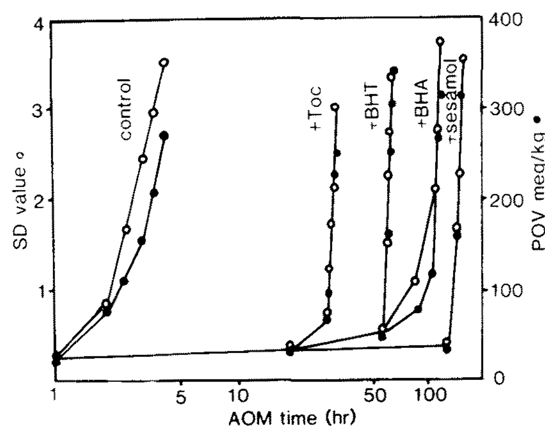


FIG. 1. Increase in sesamol dimer (SD) value and peroxide value (POV) in the active oxygen method-oxidized methyl oleate containing 0.05% α-tocopherol (toc), BHT, BHA and sesamol. One SD value was defined as 1.0 of absorbance at 550 nm against 100 mg of the fatty ester.

EXPERIMENTAL PROCEDURES

Materials. Sesamol dimer (I) was prepared as described elsewhere (8,9). Carbonmonoxy hemoglobin (COHb) was prepared by bubbling CO gas into partially purified human oxyhemoglobin solution in 0.1 M phosphate buffer (pH 7.0). Concentration of the hemoglobin was determined spectrophotometrically on a heme basis by use of a molecular extinction coefficient: 13,400 at 569 nm (10). Methyl oleate (purity 72%) and methyl linoleate (purity >95%) were from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Purified soybean oil was from Showa Sangyo Co. (Tokyo, Japan). Japan Pharmacopoeia sesame oil free from sesamol was used. Lard was prepared for use from fresh hog subcutaneous tissue by pressing and washing in hot distilled water. Butylated hydroxytoluene (BHT) and butylated hydroxyanisol (BHA) were obtained from Nikki-Universal Co. (Tokyo, Japan). dl-α-Tocopherol (toc) was a product from Tokyo Kasei Kogyo Co. Sesamol was a product of Aldrich Chemical Co. (Milwaukee, Wisconsin), which was recrystallized from chloroform-petroleum ether before use.

Oxidation of lipid samples with or without antioxidants. Active oxygen method. Oils or fats (methyl oleate, soybean oil, sesame oil and lard) with or without 0.05% antioxidant were autoxidized by the active oxygen method (AOM) (11). A 20-ml portion of each sample was placed in a tube and aerated with purified air at the rate of 2.3 ml/sec and at 98 C.

Incubation at 50 C. About 2 g of methyl linoleate with or without 0.05% antioxidant was incubated in a loosely covered dish (diameter 40 mm).

Ultraviolet irradiation. About 2 g of methyl oleate or methyl linoleate containing 0.05 or 1.0% antioxidant was irradiated by ultraviolet light at room temperature and at a distance of 40 cm from two ultraviolet lamps (325 nm) (Toshiba Co.).

Sesamol dimer (SD) method. A reaction mixture of 0.60 ml of 2-propanol solution containing 10 mg of the autoxidized oil or fat with the antioxidant, 3.50 ml of 0.1 M phosphate buffer (pH 7.0), 0.50 ml of 3.0 mM SD (I) in acetonitrile and 0.50 ml solution containing 7.5 nmol of COHb was shaken vigorously for about 30 sec in a tube protected from light. Chloroform (5.0 ml) was added to extract the violet-colored quinone (II), and the extract was dried by filter paper to measure absorbance at 550 nm by use of a Hitachi 100-10 spectrophotometer. SD value was expressed by the

*To whom correspondence should be addressed.

TABLE 1

Induction Periods of the Substrate Oil or Fatty Ester Oxidized by Active Oxygen Method

Substrate	Antioxidant (0.05%)	Induction period (hr) to reach 1 SD value	100 meq/kg POV
Methyl oleate	None	2	3
	α-Tocopherol	28	28
	BHT	56	56
	BHA	85	90
	Sesamol	120	120
Soybean oil	None	7	7
	α-Tocopherol	8	7
	BHA	9	9
	BHT	18	16
	Sesamol	14	12
Sesame oil	None	10	9
	α-Tocopherol	11	11
	BHA	13	11
	BHT	27	27
	Sesamol	27	28
Lard	None	2	2
	α-Tocopherol	30	30
	BHT	50	50
	BHA	70	70
	Sesamol	90	90

absorbance against 100 mg of the substrate or fatty ester.

POV method. POV of the autoxidized oils and fats was determined by Wheeler's method (4) with 500 mg of samples.

RESULTS AND DISCUSSION

Fatty acid methyl esters, edible oils and fats containing antioxidants were oxidized. The degree of autoxidation of each substrate oil was determined by the SD and the POV methods.

Methyl oleate with 0.05% antioxidant was oxidized by AOM (Fig. 1). The POV of the control methyl oleate gradually increased to 100 meq/kg at 3 hr and 200 meq/kg at 4 hr and the SD value of the oil increased to 1.0 at 2 hr and 2.5 at 3 hr. The presence of α-tocopherol, BHT, BHA and sesamol delayed the increases in POV and SD value in a similar fashion. The induction periods for POV of 100 meq/kg was almost the same as those for 1 SD value (Table 1). The relative efficacy of the antioxidants estimated by the SD and the POV methods was in the order sesamol>BHA>BHT>α-tocopherol.

When soybean oil was used as substrate for the antioxidants (0.05%), the increases in SD value and POV correlated well. The induction periods for 1 SD value were similar to those for 100 meq/kg POV. Similar results were obtained for sesame oil and lard (Table 1).

Methyl linoleate containing 0.01% antioxidant was incubated at 50 C under air. The POV and SD value increased similarly with the incubation time. The relative antioxidant potency was in the order sesamol>BHT>BHA>α-tocopherol in both the SD and the POV methods (Fig. 2). When methyl linoleate containing 0.1% antioxidant was irradiated by ultraviolet light, the effects of the antioxidants were shown by the prolonged induction periods of SD value in the order sesamol>BHA>BHT>α-tocopherol (Fig. 3A). When methyl oleate with 0.05% antioxidant was irradiated, the induction periods estimated by the SD value were prolonged in the order sesamol>BHA>BHT>α-tocopherol (Fig. 3B).

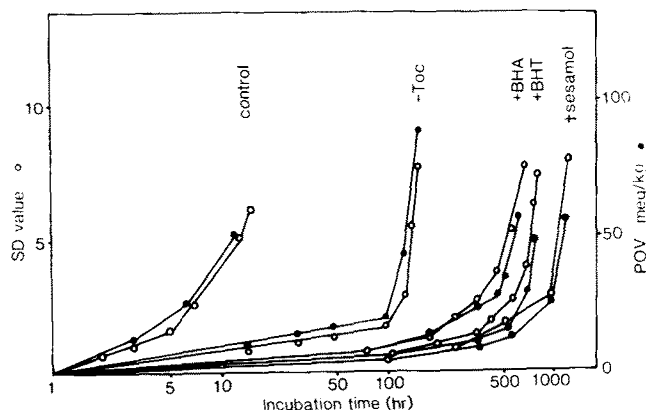


FIG. 2. Increase in sesamol dimer (SD) value and peroxide value (POV) in the 50 C-incubated methyl linoleate containing 0.05% α-tocopherol (toc), BHA, BHT and sesamol. One SD value was defined as 1.0 of absorbance at 550 nm against 100 mg of the ester.

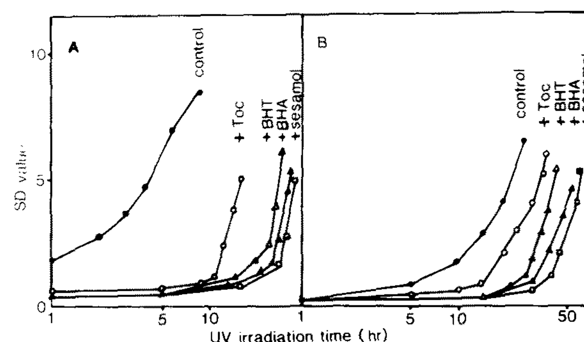


FIG. 3. Increase in sesamol dimer (SD) value in the ultraviolet irradiated methyl linoleate containing 0.1% antioxidant (A); and methyl oleate containing 0.05% antioxidant (B). One SD value was defined as 1.0 of absorbance at 550 nm against 100 mg of the ester.

These results indicate that the potency of antioxidant can be evaluated by the SD method. There were good correlations of antioxidant potency between the SD and the POV methods with different oils and the different antioxidants. While the POV method requires 500 mg of the oil to accurately determine the potency of the antioxidants, the SD method requires less than 10 mg of the substrate. Thus, the SD method is useful to estimate the potency of antioxidants with a small amount of oils or fatty esters.

REFERENCES

1. Stuckey, B.N., in *Handbook of Food Additives*, edited by T.E. Furia, The Chemical Rubber Co., Cleveland, 1968, pp. 209-245.

2. Wheeler, D.H., *Oil Soap 9*:89 (1932).
3. *Official Methods of Analysis of the Association of Official Analytical Chemists* (14th ed.) edited by S. Williams, AOAC, Arlington, VA, 1984, pp. 507.
4. Sinnhuber, R.O., T.C. Yu and T.C. Yu, *Food Res. 23*:626 (1958).
5. Gray, J.I., *J. Am. Oil Chem. Soc. 55*:539 (1978).
6. Kikugawa, K., T. Sasahara and T. Kurechi, *Chem. Pharm. Bull. 31*:591 (1983).
7. Kikugawa, K., T. Nakahara, Y. Taniguchi and M. Tanaka, *Lipids 20*:475 (1985).
8. Kurechi, T., K. Kikugawa and S. Aoshima, *Chem. Pharm. Bull. 29*:2351 (1981).
9. Kikugawa, K., Y. Ohhashi and T. Kurechi, *J. Food Hygienic Soc. Japan 23*:462 (1982).
10. Antonini, E., and M. Brunori, in *Frontiers of Biology*, edited by A. Newberger and E.C. Tatum, Vol. 21, North-Holland Publishing Co., Amsterdam, 1971, p. 19.
11. King., A.E., H.L. Roschen and W.H. Irvin, *Oil and Soap 10*:105 (1933).

[Received June 16, 1986]