Activity of Antioxidants in Solution and in Irradiated Heterogeneous System

Branka Katusin-Razem* and **Dusan Razem**
Ruder Bošković Institute, 41000 Zagreb, Croatia

The efficiency of some common antioxidants, α-tocopherol, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), was studied relative to β-carotene in a homogeneous solution and in a model system of an irradiated solid food. Relative reactivities in homogeneous solution covered a range of three orders of magnitude, α-tocopherol being the best and BHT the poorest antioxidant of the three. In irradiated systems consisting of oleic acid coated on a solid support (egg white), the range of reactivities was much narrower within one order of magnitude. In solution, there was a parallelism of the relative reactivities with oxidizing alkoxyl radicals derived from oleic acid hydroperoxides and *tert*-butyl hydroperoxide. On the solid support the relative reactivities of α-tocopherol and BHA with oleic acid radiation-induced oxidizing radicals were reversed, BHA appearing the best. Efficient antioxidants do not retain their great antioxidant activity in comparison with the moderate ones on transition from a homogeneous solution to a heterogeneous system. Relative efficiencies of antioxidants do not critically depend on the nature of oxidizing radicals in heterogeneous media.

KEY WORDS: Antioxidant activity, BHA, BHT, β-carotene, irradiated food model, lipid peroxidation, solid support, α-tocopherol.

Free-radical chain oxidation of lipids in biological materials has been related to a number of diseases (1), cancer (2) and aging (3). Although a straightforward causal relationship between lipid peroxides and pathological disorders has not been unambiguously established (4,5), the ability of antioxidants to mitigate ill effects provides circumstantial evidence for the role played by peroxides in pathology (6,7). It is not surprising, therefore, that the mechanisms of lipid peroxidation and its inhibition by antioxidants have been the subjects of numerous studies. Most quantitative studies on the effectiveness of antioxidants were concerned with homogeneous liquid systems (8) or emulsions (9), which are characterized by a good mobility of reactants.

The efficiency of antioxidants in heterogeneous systems or systems with poor mobility of reactants is far less known. The mechanisms in these systems, as a rule, are not the same as those in homogeneous or microheterogeneous media. Nevertheless, it has been shown, somewhat contrary to expectations, that the chain oxidation readily proceeds in the absence of solvent molecules (10), as well as in systems solidified by low temperatures (11), and that antioxidants can terminate the chain reaction in the dry state (10). However, little quantitative knowledge exists on the efficiency of antioxidants in radiation-induced peroxidations inside or on the surface of solid systems, which are relevant to the important technique of food preservation by irradiation (12,13).

We have studied the inhibitory effects of antioxidants on the reactions of oxidizing radicals, in solution and on a solid support, in an attempt to better understand the antioxidative action in irradiated solid systems (14). In the present work, we have tried to quantitate the effects of some common antioxidants in radiation-induced peroxidations relative to the antioxidative properties of β-carotene.

*To whom correspondence should be addressed at Ruder Bošković Institute, Bijenička cesta 54; P.O.B. 1016, 41000 Zagreb, Croatia.

MATERIALS AND METHODS

Materials. Oleic acid (OlH) (for biochemical purposes, Merck, Darmstadt, Germany); *tert*-butyl hydroperoxide (*t*-BuOOH) (purum, Fluka, Buchs, Switzerland); β-carotene (β-car) and D,L α-tocopherol (α-toc) (for biochemical purposes, Merck); $FeSO_4 \times 7H_2O$ (analytical reagent, Merck); butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) (Sigma, St. Louis, MO) were used as received. Acetone and methanol (MeOH) (analytical reagent grade; Kemika, Zagreb, Croatia) were used as received, while chloroform (analytical reagent grade, Kemika) was distilled before use. Egg white powder was supplied by Agrokomerc (Velika Kladuša, Bosnia-Herzegovina). Oleic acid hydroperoxides (OlOOH) were prepared by irradiating a thin layer of silica gel coated with oleic acid with ^{60}Co gamma rays in an open petri dish and subsequent isolation by column chromatography (15). The quantities of both *t*-BuOOH and OlOOH were assayed by spectrophotometric iodometric method (16).

Homogeneous solutions in chloroform/methanol (7:3, vol/vol) contained 1.6 mmol/dm^3 of β-carotene and the same concentration of OlOOH or *t*-BuOOH. The addition of 1.8 mmol/dm^3 of ferrous iron to this solution catalyzed the decomposition of OlOOH or *t*-BuOOH, and the formed corresponding alkoxyl free radicals subsequently bleached β-carotene. The remaining unbleached absorption of β-carotene was measured spectrophotometrically 5 min after the addition of Fe^{2+} against the solvent.

Solid model systems were prepared in the following way: Solid egg white was defatted by extraction with the mixture of chloroform and methanol (7:3, vol/vol) in a Soxhlet apparatus. OlH, β-carotene and varying amounts of antioxidants were dissolved in acetone, and the solutions were mixed with egg white in such a proportion as to attain, after evaporation of the solvent in a rotating evaporator, concentrations typical of whole egg powder (17):500 mmoles oleic acid and 0.032 mmoles β-carotene per kg of egg white solid.

Solid model systems were irradiated in the presence of air by ^{60}Co gamma rays at a dose rate of 2 kGy/h. The lipidic component was extracted into chloroform/methanol (7:3, vol/vol). The degradation of β-carotene was measured in the extract by spectrophotometry at the maximum of β-carotene absorption, 455 nm, and the remaining absorption was expressed as percentage of the control (unirradiated samples).

RESULTS

OlOOH or *t*-BuOOH alone do not bleach β-carotene in solution. The absorbance of the respective solutions is stable and is termed A_o. The addition of ferrous ions to the solutions starts the decomposition of hydroperoxides and subsequent bleaching of β-carotene by ensuing alkoxyl

Copyright © 1994 by AOCS Press

radicals. The absorbance decreases with time; at some specified time it is termed A, so that:

$$\Delta A = A_o - A \quad [1]$$

If an antioxidant (AH) is added to the solution, together with ferrous ions, the bleaching of β-carotene is slower. The absorbance A_{AH} at the same specified time, due to the protective effect of antioxidant, is higher than it would have been in the absence of antioxidant, so that the following expression applies:

$$A_o - A > A_o - A_{AH} \quad [2]$$

i.e.,

$$\Delta A > \Delta A_{AH} \quad [2a]$$

Following simple kinetic considerations and assuming the validity of some basic requirements (18), the following expression applies:

$$\Delta A_{AH} = \Delta A(k_{\beta\text{-car}}\,[\beta\text{-car}]/(k_{\beta\text{-car}}[\beta\text{-car}] + k_{AH}[AH]) \quad [3]$$

which can be easily transformed into:

$$\Delta A/\Delta A_{AH} = 1 + k_{AH}[AH]/k_{\beta\text{-car}}[\beta\text{-car}] \quad [4]$$

This expression represents the straight line $\Delta A/\Delta A_{AH}$ vs. [AH]/[β-car], which intercepts the ordinate at 1 and has a slope of $k_{AH}/k_{\beta\text{-car}}$.

Straight lines obtained by this method for α-toc, BHA and BHT are shown in Figure 1 (A–C). Rate constants for the reactions of oleic acid alkoxyl radicals (AlO•) and *t*-butoxyl (*t*-BuO•) radicals with antioxidants relative to the rate constant for the reaction of the same radical species with β-car, all in $CHCl_3$/MeOH solution, are given in Table 1.

The irradiation of egg white support coated with β-car alone does not produce an appreciable decrease of the β-car absorbance as subsequently measured in the extract. The irradiation of solid support coated with both oleic acid and β-car results in the decrease of β-car absorption. Analogously to the situation in solutions, whereby the absorbance decreased with time, in solid irradiated systems the absorbance decreased with dose. This decrease was first-order with respect to dose, i.e., it had the form: $\ln(A/A_o) = -kD$ with a "rate constant" of k = 0.02 $(kGy)^{-1}$ in the absence of OlH and 0.77 $(kGy)^{-1}$ in the presence of OlH (not shown). Equation 1 holds at any specified dose. Depending on the amount of the antioxidant present in the coating, the decrease of the β-car absorption varied, but Equation 2 held in those situations as well. Radiation-induced degradation of β-car on solid support in the presence of α-toc, BHA and BHT is shown in Figure 2 (A-C). The expression formally analogous to Equation 4 can be written for the situation encountered with solid support, with the understanding, however, that the concept of the homogeneous rate constants does not apply—reaction efficiencies κ must be used instead of *k*'s.

The ratios of antioxidant efficiencies relative to β-car have been calculated from the readings of A_o, A and A_{AH} from Figure 2 at dose intervals of 0.5 kGy, and by calculating the slopes of the straight lines (Equation 4) through point 1 on the ordinate. Relative activity of α-toc was calculated for the antioxidants' concentration ratio [α-toc]/[β-car] = 0.92 and 9.2 (≈1:1 and 10:1), that of BHA for the antioxidants' concentration ratios [BHA]/[β-car] = 1.1 (≈1:1), while that of BHT was calculated for antioxidants'

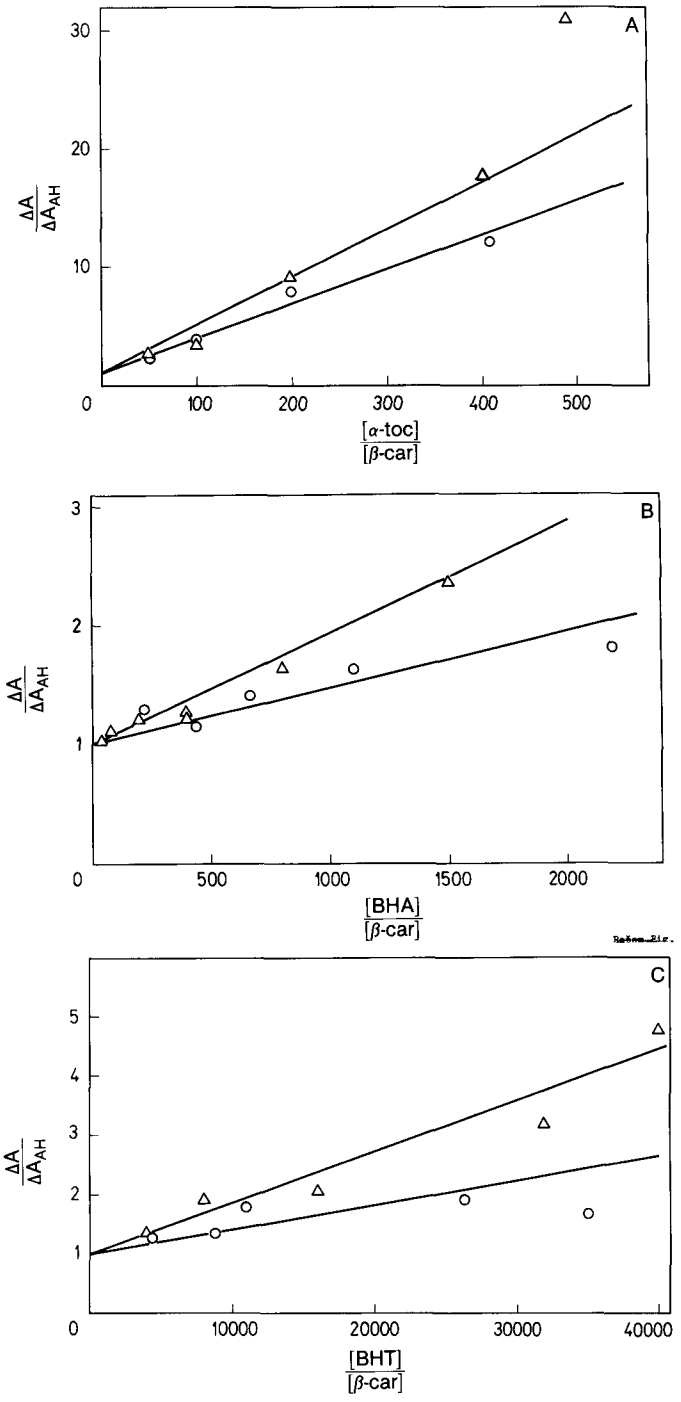

FIG. 1. Competition between antioxidants AH and β-carotene (β-car) in $CHCl_3$/MeOH (7:3, vol/vol) solutions for *t*-BuO• (Δ) and OlO•(○); A, (α-toc); B, butylated hydroxyanisole (BHA); C, butylated hydroxytoluene (BHT). Each point is an average of at least three measurements.

concentrations ratios 1.1, 10.9 and 54.5 (≈1:1, 10:1 and 50:1, respectively). The obtained values are shown in Table 1.

Basic requirements (18) involved in the derivation of Equation 4 were also valid in the systems on solid support, namely: (i) Only β-car absorbed at the wavelength of observation; (ii) the observed absorption change was stable for a reasonable period of time; and (iii) the concentration of the reference substance, β-car, was low

TABLE 1

Activity of Antioxidants Relative to β-Carotene

Antioxidant[b]	Oleic acid[a] $CHCl_3$/MeOH $k_{AH}/k_{\beta\text{-car}}$	Oleic acid[a] Solid support $\varkappa_{AH}/\varkappa_{\beta\text{-car}}$	t-BuOOH[a] $CHCl_3$/MeOH $k_{AH}/k_{\text{b-car}}$
α-Toc	$(3.0 \pm 0.5) \times 10^{-2}$	1.6 ± 0.3	$(4.1 \pm 1.5) \times 10^{-2}$
BHA	$(4.8 \pm 1.4) \times 10^{-4}$	2.8 ± 0.6	$(1.9 \pm 0.9) \times 10^{-3}$
BHT	$(4.1 \pm 1.7) \times 10^{-5}$	0.5 ± 0.1	$(8.6 \pm 2.0) \times 10^{-5}$

[a]Source of oxidizing radicals.
[b]Abbreviations: α-toc, α-tocopherol, BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; β-carotene, β-car; t-BuOOH, *tert*-butyl hydroperoxide.

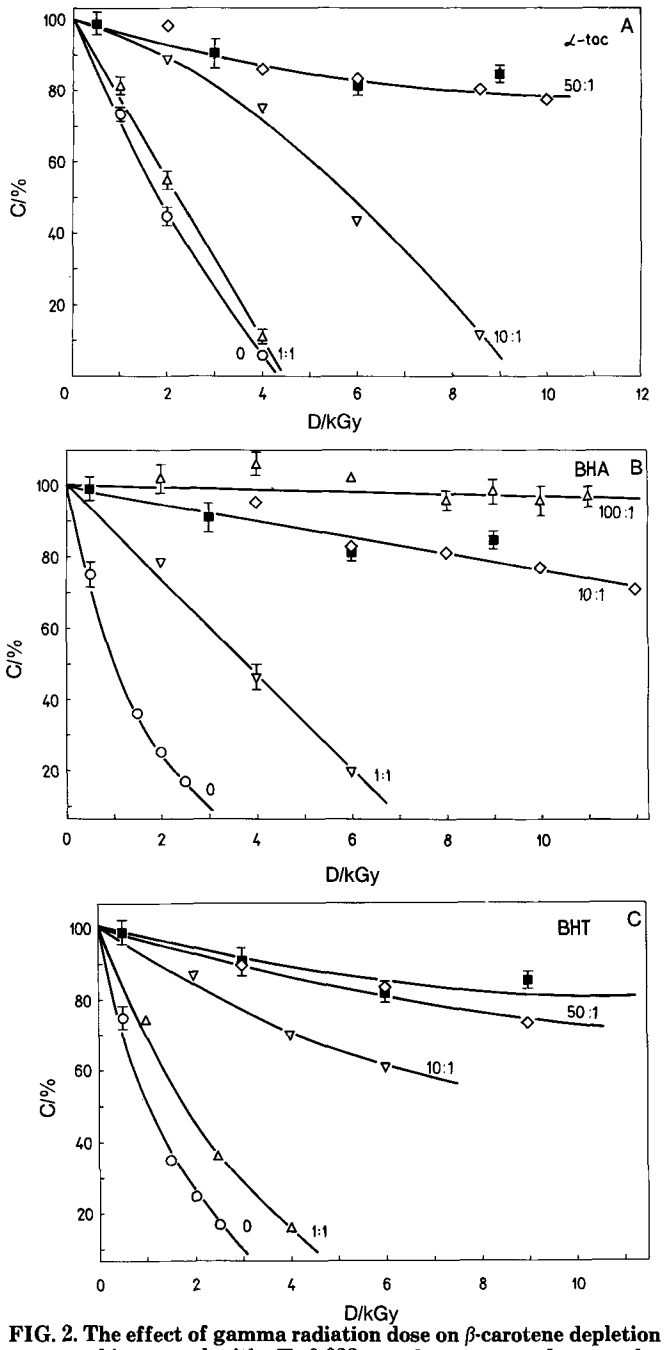

FIG. 2. The effect of gamma radiation dose on β-carotene depletion on egg white coated with: ■, 0.032 mmol β-carotene alone per kg egg white; ○, 500 mmol oleic acid/kg egg white in addition to β-car; Δ, = ∇ and ◇, AH added to the previous mixtures (β-car + oleic acid) so that various concentration ratios AH/β-car were achieved, as marked on the curves; A, α-toc; B, BHA; C, BHT. Each point is an average of at least three measurements. Abbreviations as in Figure 1.

enough so that the first-order decrease of its absorption with dose could be followed over more than three half-dose periods, down to 10% of the initial value. The bimolecular nature of the bleaching reaction, however, had to remain in the realm of an assumption because there is no way to test it in a solid system.

The assumption that no other competing processes interfere with the observed reactions of oxidizing radicals with antioxidants in solid systems was tested in the following way. Allowance was made for the possibility that oxidizing radicals reacted also by an unspecified reaction, characterized by $\varkappa$, in addition to their reactions with antioxidants. This possibility was accounted for by adding an additional term $\varkappa$ in the denominator of an equation analogous to Equation 3. The transformation of that equation containing the additional term gives:

$$\Delta A/\Delta A_{AH} = 1 + \varkappa/\varkappa_{\beta\text{-car}}[\beta\text{-car}] + \varkappa_{AH}[AH]/\varkappa_{\beta\text{-car}}[\beta\text{-car}] \quad [5]$$

Equation 5 describes a straight line $\Delta A/\Delta A_{AH}$ vs. $[AH]/[\beta\text{-car}]$ (at a constant concentration of β-car), which has an intercept $1 + \varkappa/\varkappa_{\beta\text{-car}}[\beta\text{-car}]$ and a slope $\varkappa_{AH}/\varkappa_{\beta\text{-car}}$. The magnitude of the intercept was calculated by extrapolation to zero of the pairs of data for $\Delta A/\Delta A_{AH}$ at the used ratios of concentrations of antioxidants, $[AH]/[\beta\text{-car}]$ (two pairs for α-toc, three pairs for BHT). The values for the intercept were 0.60 with α-toc and 0.92 with BHT. Within the experimental error, both values are close to 1, which means that $\varkappa$ is close to zero.

DISCUSSION

In homogeneous solutions, relative reactivities of antioxidants do not depend on the kinds of solvents used (19). Furthermore, relative reactivities of tocopherol antioxidants in homogeneous solutions also do not depend on the kinds of oxyradicals used (substituted phenoxyl and peroxyl radicals) (20). Present results show that the latter is also true for antioxidants that are not members of the same homologous series; antioxidant activities of α-toc, BHA and BHT relative to β-car were approximately of the same order with alkoxyl radicals derived from OlH as with *tert*-butyl alkoxyl radicals. Actually, antioxidant activities, relative to β-car, of these antioxidants were about twice as large with t-BuO•, as compared to OlO• radicals (Table 1). Relative reactivities of the most reactive α-toc and least reactive BHT spanned three orders of magnitude in $CHCl_3$/MeOH homogeneous solutions (Table 1).

A similar range of reactivities of the same three antioxidants was measured with different radicals in different solvents (8,21-25). Because β-car was not used in those studies, relative antioxidant activities were compared

TABLE 2

Relative Activities of Antioxidants in Various Media (the activity of BHA = 1 where available)[a]

Medium	Source of oxidizing radicals	Relative activity			Reference number
		α-toc	BHA	BHT	
H_2O soln.	t-BuOOH		1	0.95	8
H_2O soln.	13-LOOH		1	0.59	8
H_2O soln.	Tryptophane		1	0.021	21
$CHCl_3$/MeOH	OlOOH	62.5	1	0.085	This work
$CHCl_3$/MeOH	t-BuOOH	21.6	1	0.045	This work
C_6H_6/EtOH	Tocopherol	15.2		0.048	22
C_6H_6 soln.	Subst. phenoxyl	90		0.084	23
CCl_4	CCl_3OOH	4.6	1	0.016	24
CCl_4	CCl_3•	0.6	1	0.001	24
Cyclohexane	$C_6H_{11}OOH$	2.3	1	0.003	24
Chlorobenzene	styrene	30	1	0.15	25
SDS micelle	LOOH	2.4	1	1.4	26
SDS micelle	LOOH	3.7	1	1.1	9
Liposomes	superoxide	1	1	1	27
Oleic acid on egg white	OlOOH	0.6	1	0.2	This work
Cottonseed oil on gelatin	OlOOH + LOOH	14.6	1	0.4	28
Lecithin thin film	OlOOH	4		0.1	10
Tributyrin at 170 K	BuOOH	3.1	1	9.9	11
Triolein at 190 K	OlOOH	2.2	1	>2.3	11
Trilinolein at 166 K	LOOH	1.1	1	2.3	11

[a]Abbreviations as in Table 1; soln., solution; OlOOH, oleic acid hydroperoxides; LOOH, linoleic acid hydroperoxides.

internally, taking the reactivity of BHA = 1. Our results from Table 1, recalculated in this way, are entered in Table 2, together with some results from the literature, recalculated accordingly and also entered in Table 2 for comparison. In homogeneous solutions, the reactivity of the most reactive α-toc was several hundred times larger than that of the least reactive BHT, both according to this work and the literature data (22–25).

On transition from a homogeneous solution to various heterogeneous systems, there is agreement in the literature that the antioxidant activity of α-toc is reduced in dilinoleylphosphatidylcholine (DLPC) bilayers (29), membranes of egg phosphatidylcholine (PC) liposomes (30), DLPC liposomes (31) and soybean PC liposomes (32). In spite of the reduced antioxidant activity in heterogeneous systems, α-toc was efficient at low concentrations in inhibiting autoxidation of linoleic acid in monolayers (33), and in human erythrocyte ghosts (34); autoxidation proceeded in those systems only after the consumption of all available α-toc.

However, not only was the absolute activity of α-toc reduced in heterogeneous systems (29–32), the relative activities of antioxidants in heterogeneous systems also spanned a much narrower range, as compared to three orders of magnitude in homogeneous solutions. In sodium dodecylsulfate (SDS) micelles, the activity ratio α-toc/BHT was only about 2–3 (9,26). In layered systems, consisting of thin films, relative activities of α-toc/BHT were about 40 (10). In phospholipid liposomes, relative activities of all three antioxidants used (α-toc, BHA and BHT), were the same (27). In bulk solid systems, the order of relative activities was reversed, BHT being more efficient than α-toc (11). The same reversed order of activity was also observed in animal fats and bulk fatty acids (35,36). The efficiency ratio α-toc/BHT in those systems was between 1 and 0.3. In our system, coated on the solid support, the activity ratio α-toc/BHT was three, the same as the value obtained in SDS micelles and of the same order of magnitude as the values in other heterogeneous systems (Table 2).

It should be kept in mind that all antioxidant activity data presented here are relative to β-car, which served to visualize the extent of antioxidants' actions in homogeneous, as well as in heterogeneous, systems. Because of the restricted mobility of all reacting species in heterogeneous systems, it is difficult to interpret the data in those systems. Specific orientation of molecules on the solid support might alter their reactive properties. The activity of β-car can only be regarded with difficulty as a standard, because the microenvironment is expected to influence β-car activity in a different way than it would influence the activities of other antioxidants used.

The reduced antioxidant activity of α-toc relative to β-car in heterogeneous systems can be partly explained by the decreasing ability of α-toc to inhibit lipid peroxidation with decreasing partial pressure of oxygen, while the antioxidant activity of β-car increases with decreasing p_{O_2} (37). These opposing tendencies would bring relative antioxidant activities of α-toc and β-car closer to each other, as has already been found in rat liver microsomes (38). Reduced p_{O_2} would especially exist in bulk fatty acids, triglycerides and natural fats.

Solid support may exert an additional effect on the relative activities. It was hypothesized that hydrogen bonding between the chromanol-nucleus hydroxyl of α-toc and the phosphate oxygen of phospholipids might partly reduce the accessibility of the antioxidative functional OH group of the vitamin to the fatty acid acyl chains (39). Similar interactions with proteinaceous support would also reduce the range of antioxidant activities on egg white in our case.

The increase of the activity of BHA on egg white support, relative to both α-toc and BHT, found in this study might perhaps also be explained by the ability of the BHA molecule to interact with the support with its two polar ends, one of the adsorbed positions exposing the antioxidative OH group, whereas the interactions of both α-toc and BHT would invariably make their OH groups inaccessible.

In conclusion, it can be stated that differences among efficient antioxidants are not large in the solid state. Furthermore, relative antioxidant efficiencies do not critically depend on the nature of the oxidizing radicals. This has practical ramifications in the selection of antioxidants for food processing (40)—synthetic, less costly antioxidants can be used in food irradiation processing to prevent radiation-induced oxidations in irradiated foods and feeds that contain lipids.

ACKNOWLEDGMENT

The skillful technical assistance of M. Rajković is gratefully acknowledged.

REFERENCES

1. Yagi, K., *Chem. Phys. Lipids 46*:337 (1987).
2. O'Brien, P.J., in *Lipid Peroxides in Biology and Medicine*, edited by K. Yagi, Academic Press, Orlando, 1982, p. 317.
3. Hirai, S., K. Okamoto and M. Morimatsu, *Ibid.*, p. 305.
4. Halliwell, B., in *Proceedings of the Upjohn Symposium on Oxidants and Disease*, edited by B. Halliwell, Federation of American Societies for Experimental Biology, Bethesda, 1989, p. 358.
5. Schaich, K.M., and H.S. Black, *Lipids 23*:568 (1988).
6. Goldstein, B.D., and G. Witz, *Free Rad. Res. Commun. 11*:3 (1990).
7. Logani, M.K., and R.E. Davies, *Lipids 15*:485 (1980).
8. Bors, W., C. Michel and M. Saran, *Biochim. Biophys. Acta 796*:312 (1984).
9. Pryor, W.A., T. Strickland and D.F. Church, *J. Am. Chem. Soc. 110*:2224 (1988).
10. Agarwal, S., and S.N. Chatterjee, *Radiat. Res. 100*:257 (1984).
11. Zhu, J., W.J. Johnson, C.L. Sevilla, J.W. Herrington and M.D. Sevilla, *J. Phys. Chem. 94*:7185 (1990).
12. Schaich, K.M., *CRC Crit. Rev. Food Sci. Nutrit. 13*:189 (1980).
13. Wills, E.D., *Int. J. Radiat. Biol. 37*:403 (1980).
14. Katusin-Razem, B., B. Mihaljević, and D. Razem, *J. Agric. Food Chem. 40*:662 (1992).
15. Frankel, E.N., C.D. Evans, H. Moser, D. McConnel and J.C. Cowan, *J. Am. Oil Chem. Soc. 38*:130(1961).
16. Hicks, M., and J.M. Gebicki, *Anal. Biochem. 99*:249 (1979).
17. Bergquist, D.H., in *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 8, 3rd edn., Wiley, New York, 1979, p. 429.
18. Bors, W., C. Michel and M. Saran, in *CRC Handbook of Methods for Oxygen Radical Research*, edited by R.A. Greenwald, CRC Press, Boca Raton, 1985, p. 181.
19. Mukai, K., S. Kikuchi and S. Urano, *Biochim. Biophys. Acta 1035*:77 (1990).
20. Mukai, K., K. Okabe and H. Hosose, *J. Org. Chem. 54*:557 (1989).
21. Richards, J.T., and F.A. Adams, *Internat. J. Food Sci. Technol. 22*:501 (1987).
22. Mukai, K., N. Nishimura, A. Nagano, K. Tanaka and E. Niki, *Biochim. Biophys. Acta 993*:168 (1989).
23. Mukai, K., K. Daifuku, S. Yokoyama and M. Nakano, *Ibid. 1035*:348 (1990).
24. Simic, M.G., and E.P.L. Hunter, in *Radioprotectors and Anticarcinogens*, edited by O.F. Nygaard, and M.G. Simic, Academic Press, New York, 1983, p. 449.
25. Burton, G.W., and K.U. Ingold, *J. Am. Chem. Soc. 103*:6472 (1981).
26. Castle, L., and M.J. Perkins, *Ibid. 108*:6381 (1986).
27. Janero, D.A., N. Cohen, B. Burghardt and B.H. Schaer, *Biochem. Pharmacol. 40*:551 (1990).
28. Togashi, H.J., A.S. Henick and R.B. Koch, *J. Food Sci. 26*:186 (1961).
29. Barclay, L.R.C., K.A. Baskin, S.J. Locke and M.R. Vinquist, *Can. J. Chem. 67*:1366 (1989).
30. Niki, E., M. Takahashi and E. Komuro, *Chem. Lett.*:1573 (1986).
31. Barclay, L.R.C., A.M.H. Bailey and D. Kong, *J. Biol. Chem. 260*:15809 (1985).
32. Niki, E., *Br. J. Cancer 55, Suppl. VIII*:153 (1987).
33. Wu, G.S., R. Stein and J.F. Mead, *Lipids 12*:971 (1977).
34. Yamamoto, Y., E. Niki, J. Eguchi, Y. Kamiya and H. Shimasaki, *Biochim. Biophys. Acta 819*:29 (1985).
35. Cort, W.M., *J. Am. Oil Chem. Soc. 51*:321 (1974).
36. Szelag, H., Z. Dlugokecki and W. Zwierzykowski, *Fat Sci. Technol. 92*:310 (1990).
37. Burton, G.W., and K.U. Ingold, *Science 224*:569 (1984).
38. Vile, G.F., and C.C. Winterbourn, *FEBS Lett. 238*:353 (1988).
39. Stocker, R., Y. Yamamoto, A.F. McDonagh, A.N. Glazer and B.N. Ames, *Science 235*:1043 (1987).
40. Löliger, J., in *Free Radicals and Food Additives*, edited by O.I. Aruoma, and B. Halliwell, Taylor and Francis, London, 1991, p. 121.

[Received June 23, 1993; accepted February 10, 1994]