Mackerel Skin Lipids as an Unsaturated Fat Model System for the Determination of Antioxidative Potency of TBHQ and Other Antioxidant Compounds

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ABSTRACT AND SUMMARY

A comparative study on the activity of antioxidation of butylhydroxy anisole, (BHA), butylhydroxy toluene (BHT), *tert*-butylhydroquinone (TBHQ), α-tocopherol, and tempeh oil has been investigated by using the readily oxidizable mackerel skin lipids as the tested model system. The oxidation rate of the tested lipids was mainly followed by measuring the weight gain, but some peroxide value, thiobarbituric acid (TBA), and free fatty acid (FFA), and the changes of composition of fatty acids and carbonyls were also determined and used for some practical evaluation of the various influences of these antioxidatns. Overall, the order of effectiveness for inhibiting the oxidation in mackerel skin lipids has found to be TBHQ > α-tocopherol > tempeh oil > BHA > BHT at the concentrations of 0.02% for all synthetic compounds, and 0.1% and 5% for α-tocopherol and tempeh oil, respectively. In addition, TBHQ has demonstrated not only to be the most powerful antioxidant for the unsaturated marine lipids but also to retard the formation of FFA and carbonyls from lipid hydrolysis and secondary oxidation reactions. The superior effectiveness of TBHQ in the highly unsaturated mackerel skin lipids is especially noteworthy in order to improve oxidative stability of marine oils and some fatty fishes.

INTRODUCTION

The oxidation of lipids takes place in a series of steps and is often referred to as a "free radical" type of oxidation because the initial step is the formation of a free radical (1,2). The free radicals formed in lipid oxidation act as strong initiators of further oxidation; hence, the oxidative reaction in various lipids is often described as an autocatalytic process (3,4).

Among the commonly used antioxidants, three [butylhydroxy anisole (BHA), butylhydroxy toluene (BHT), and propyl gallate (PG)] are of inadequate potency in some highly unsaturated lipids such as marine oils (5,6). However, *tert*-butylhydroquinone (TBHQ) has been found to exhibit excellent potential in crude oils and fatty foods (7,8). Favorable results have been obtained in tests evaluating TBHQ in marine oils (9,10) and seed oils (11). Test feeding and comparative biochemical studies have shown TBHQ to be safe for food use (12). In this report, the effectiveness of TBHQ in marine lipids, in comparison with other synthetic and natural antioxidant compounds, has been evaluated by using a readily oxidizable model system, mackerel skin lipids (4,13).

EXPERIMENTAL PROCEDURES

Preparation of Mackerel Skin Lipids

Mackerel skin lipids (total 3 kg), extracted by the method of Bligh and Dyer (13), were pooled from 20 batches of skin samples (including the fat layer underneath the skin). Skins, lipid content 46%, had been separated manually from the fillets of Atlantic mackerel (*Scomber scrombrus*) within a few hours of their being landed at the dock in Nova Scotia in September, 1975. Analytical data for skin lipids were: free fatty acid (FFA) %, 0.2; thiobarbituric acid (TBA) molar value, 14 μmole/kg oil; peroxide value (PV), 0; and iodine value (IV), 121. Data for the meat lipids extracted from the same fillets after the skin was removed are also listed here for comparison and were: lipid content, 17%; FFA%, 1.3; IV, 127; and TBA, 3.2 μmole/kg fish. The lipids were stored in brown glass bottles under nitrogen and held at -25 C before use.

Preparation of Antioxidant Reagents

The following antioxidants of food grade have been used in this research: BHA and BHT, from ICN Pharm. Inc., Plainview, NY; TBHQ, PG, and *d*-α-tocopherol (α-tocoph) from Eastman Kodak Co., Rochester, NY; and Tenox-22 (containing 20% BHA, 6% TBHQ, 4% citric acid, and 70% propylene glycol) from Eastman Chem. Products, Inc., Kingsport, TN. Citric acid (CA) and propylene glycol of ACS grade from Fisher Sci. Co., and refined corn oil, were used for preparing stock reagent solutions of other antioxidants. Tempeh oil used in this study was extracted by Bligh and Dyer's method (14) from tempeh, a fermented soybean from Indonesia which had previously shown some inhibition of oxidation (15,16).

Tempeh oil, α-tocopherol, and Tenox-22 were added directly to the model lipids for testing. Three stock reagents containing, respectively, 20% BHT, 10% BHT & 10% BHA, and. 10% BHT & 10% TBHQ, prepared by using corn oil as solvent, were designated sample Nos. B, D-1, and D-2, respectively (Table I). Stock solutions were also prepared by dissolving BHA and TBHQ in propylene glycol in the following concentrations and designated: A, 20% BHA; A-1, 20% BHA + 5% CA + 5% PG; C, 20% TBHQ; C-1, 20% TBHQ, + 5% CA + 5% PG; and D-3, 10% BHA + 10% TBHQ.

Measurement of Antioxidation Activity

The basic approach was the weight gain method described in an earlier report (4). After adding the antioxidant to the tested mackerel skin lipids (about 10 g) in a glass petri dish (9 cm ID) and mixing, the whole was accurately weighed. The samples were then placed in a forced hot air oven at 60 C, and the rates of oxidation in terms of weight increase were recorded daily. PV, TBA molar values, and other analyses also were determined on parallel samples to also evaluate specific reactions.

Analytical Procedures

PV and IV were determined by the AOCS official methods, Cd 8-53 and Tg 1-63, respectively. FFA content was measured by using an improved titrimetric method

TABLE I

Comparison of Effectiveness of Various Antioxidants by Determining the Time Required of the Induction Period and of the Samples with 1% Weight Increases at 60 C Using Mackerel Skin Lipids as a Model System[a]

Sample Number	Antioxidant	Induction period (day)	1%, Wt increase		
			Day	PV(meq./kg Oil)	TBA(μmole/kg oil)
S (control)	none	1.5	4.5	117	310
A	0.02% BHA	3.5	8.0	98	620
A-1	0.02% BHA (with CA & PG)	3.5	---	---	---
B	0.02% BHT	1.6	4.2	112	325
C	0.02% TBHQ	16	28	105	(360)[b]
C-1	0.02% TBHQ (with CA & PG)	16	---	---	---
D-1	0.01% BHA + 0.01% BHT	3.2	6.5	---	440
D-2	0.01% BHT + 0.01% TBHQ	10.5	---	---	---
D-3	0.01% BHA + 0.01% TBHQ	10.5	---	---	---
D-4	Tenox 22	6.0	13	---	---
F-1	0.1% α-tocoph	4-6	12	109	306
F-1a	0.5% α-tocoph	2-4	---	---	---
F-2	5% tempeh oil	3-5	10	120	490
F-2a	10% tempeh oil	7-10	---	---	---

[a]Abbreviations: PV = perotide value, TBA = thiobarbituric acid, BHA = butylhydroxy anisole, CA = citric acid, PG = propyl gallate, BHT = butylhydroxy toluene, TBHQ = *tert*-butylhydroquinone, and α-tocoph = α-tocopherol.

[b]The estimated value.

(17). Compositional changes in fatty acids and volatile carbonyls were evaluated quantitatively by gas liquid chromatographic (GLC) methods (6). Molar TBA values were obtained by using the modified procedure reported in 1975 by Asakawa et al. (18). Briefly, to 20 mg of the oxidized lipid sample were added 1 ml of trichloroacetic acid (35%) and 2 ml of TBA reagent (0.36% TBA + 0.1% Na_2SO_3) in a test tube (150 x 15 mm) with screw cap, and this was heated in a boiling water bath for 15 min. After the solution cooled to room temperature, 1 ml of glacial acetic acid and 2 ml of chloroform were added. The clear pink solution was used for the estimation of TBA molar value at 532 nm with standardization against 1,1,3,3-tetraethoxypropane.

RESULTS AND DISCUSSION

A primary purpose of using antioxidants in lipids is to delay a significant accumulation of free radicals and thus to improve oxidative stability. Comprehensive disucssions on the theoretical and practical aspects of various antioxidants have been reviewed recently by Sherwin (19) and Hardy (3), although antioxidant technology in food fats and oils began in the 1940s with phenolic antioxidants which were found to have outstanding stabilization properties for many fatty materials. However, we have not yet explained satisfactorily why mackerel skin lipid is extremely susceptible to oxidation, even for a marine oil (4,15). This property could be due to lack of a natural material capable of inactivating the initial free radicals. Irrespective of the reason, this particular property could simplify comparisons among added antioxidants by removing competing factors.

The inhibitory effects on oxidation of mackerel skin lipids of various synthetic and natural antioxidants, as determined by measurement of the weight gain at 60 C, are compared in Figure 1. In general, the order of antioxidation activity of these synthetic compounds was found to be TBHQ > BHA > BHT, as shown by the curves C, A, and B in Figure 1-a. No oxidation of the test mixture of skin lipids with 0.02% TBHQ could be detected after 8 days of investigations. This result agrees with previous reports (3,10,18,19) that TBHQ provided the greatest protection against autoxidation of marine lipids. It has been recognized as one of the most effective antioxidants for lipids rich in polyunsaturated fatty acids (7,8). Citric acid and propyl gallate, mixed with BHA (Curves A and A-1, Fig. 1-b) or TBHQ (Table I), respectively, only slightly enhance the antioxidation activity of these antioxidants alone in mackerel skin lipids.

Mixtures of antioxidants, combined to concentrations of 0.02% of the test lipids, have also been studied, and the results for D-3 and D-4 as plotted in Figure 1-c also indicate clearly that TBHQ is the most effective antioxidant for mackerel skin lipids at the reaction condition of 60 C. The antioxidative activity of TBHQ over the concentration range of 0.003 to 0.022% of the mackerel skin lipids was also evaluated. Changes of the rates for 7 different samples over a test period of 17 days have been plotted in Figure 2. Both the induction time and the total changes in lipid oxidation are related to the concentration of TBHQ used in the model lipid system. The maximum concentration of TBHQ permitted in edible oils under U.S. regulations is 0.02%, and this level is recognized as being optimum for achieving good oxidative stability on oils of low to medium unsaturation. However, our results showed that autoxidation in mackerel skin lipids (IV 121) can be equally well inhibited even at less than half of the allowed level (Curve 2, 0.008%, Fig. 2), and this still can give a better oxidative stability than obtained with the tests using 0.02% of BHA or BHT (Fig. 1-a, Curves A and A-1).

As well as evaluating synthetic compounds in the system based on highly unsaturated marine lipids, α-tocopherol and tempeh oil also have been tested, and their antioxidative abilities are shown in Figure 1-d. α-

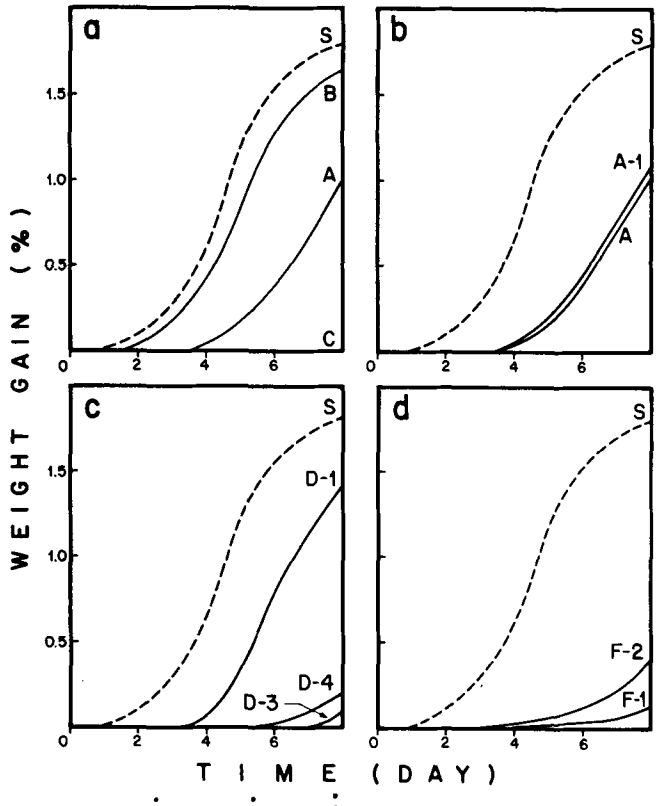

FIG. 1. Antioxidative effects on the oxidation of mackerel skin lipids mixed with various antioxidants. S (broken line) for the control sample; In (1-a), A – with 0.02% BHA, B – 0.02% BHT, and C – 0.02% TBHQ; In (1-b), A – with 0.02% BHA, and A-1, – 0.02% BHA + CA & PG; In (1-c), D-1 – 0.01% of BHA & BHT, D-3 – 0.01% of BHA & TBHQ, and D-4 – Tenox 22; In (1-d), F-1 – with 0.1% α-tocopherol and F-2 – with 5% tempeh oil. Abbreviations: BHA = butylhydroxy anisole, BHT = butylhydroxy toluene, TBHQ = *tert*-butylhydroquinone, CA = citric acid, PG = propyl gallate.

Tocopherol, a widely-used antioxidant, has been reported as showing some advantages in application to marine lipids (20,21). Tempeh, a fermented soybean preparation widely used in Southeast Asia, has been reported to contain one or more active antioxidant compounds in the oil fraction (15,16). Both α-tocopherol (0.1%) and tempeh oil (5%) have high activities in the retardation of autoxidation of mackerel skin lipids. The results for this test lipid mixed with α-tocopherol or tempeh oil at the concentration described were even better (Fig. 1) than those using BHA or BHT at the highest levels of 0.02%. A slower transition in changing from the initiation to the propagation stage during the course of oxidation (Fig. 1-d and Table I) can be deduced from the results when the natural antioxidants used in most of the present investigations are compared with the synthetic mateirlas. However, TBHQ was still indicated as the most effective and potent antioxidant for the highly unsaturated lipids of mackerel skin, even when the concentrations of the natural antioxidants were increased to 0.5% for α-tocopherol and 10% for tempeh oil (Table I). Since numerous natural compounds and mixtures are indicated to have various degrees of antioxidative activities (22-26), it is believed that more research in this field should be encouraged. The highly unsaturated and rapidly oxidized mackerel skin lipids can be recommended for use as a model system for determining antioxidant activity and studying kinetic effects (4,13,27).

The times required for the induction periods and the sample to achieve 1% weight increase have been estimated, respectively, from the reaction curves (4), and these results were used for comparing the antioxidative effectiveness of various antioxidants and mixtures as listed in Table I. The PV and TBA data of the samples with 1% weight-gain have been also listed in Table I, permitting a preliminary assessment of the possible effects on the formation of primary and secondary oxidation products from these antioxidants (13,19). From this approach, it can again be concluded that for marine lipids of those tested, TBHQ has been found to be the most powerful antioxidant. However, α-tocopherol and tempeh oil have also been shown to be effective in preventing autoxidation in this highly unsaturated lipid, and at high concentrations are even better than BHA and BHT at the permitted levels of 0.02%. PV values in all samples of 1% weight-gain were remarkably similar. The TBA molar value in the sample with BHA added was almost twice as high as the control and the samples with using other antioxidants or mixtures. But in the sample lipid mixed with 5% tempeh oil, the TBA molar value increased about 50% only above the control. However, this was still much lower than the BHA treated lipids.

Analytical results for mackerel skin lipids mixed with BHA, TBHQ, α-tocopherol, and tempeh oil, respectively,

TABLE II

Comparison of Some Chemical Data of the Model Lipids Oxidized at 60 C for 8 Days in Presence of Various Antioxidants[a]

Analysis	Control[b]	0.02% BHA	0.02% TBHQ	0.1% α-Tocoph	5% Tempeh oil
%, Wt increase	1.8	1.0	0	0.1	0.4
PV (meq/kg oil)	185	98	0	7	36
TBA molar value (μmole/kg oil)	520(14)	620	70	140	310
%, FFA	2.9(0.2)	2.0	0.5	2.5	---
Fatty Acid Composition, mole%					
saturates	36(27)	32	28	---	---
monoenes	51(44)	48	48	---	---
polyenes (excl. trienes)	9(25)	17	20	---	---
polyene Index[c]	0.25(0.93)	0.53	0.72	---	---
Carbonyls (moles/g oil x 10^{-9})					
alkanals	480	540	46	---	---
alkenals	65	160	5	---	---

[a]Abbreviations: BHA = butylhydroxy anisole, TBHQ = *tert*-butylhydroquinone, α-tocoph = α-tocopherol, PV = peroxide value, TBA = thiobarbituric acid, FFA = free fatty acid.

[b]Analytical data for the unoxidized lipids were shown in parentheses.

[c]Polyene index = polyenes/saturates.

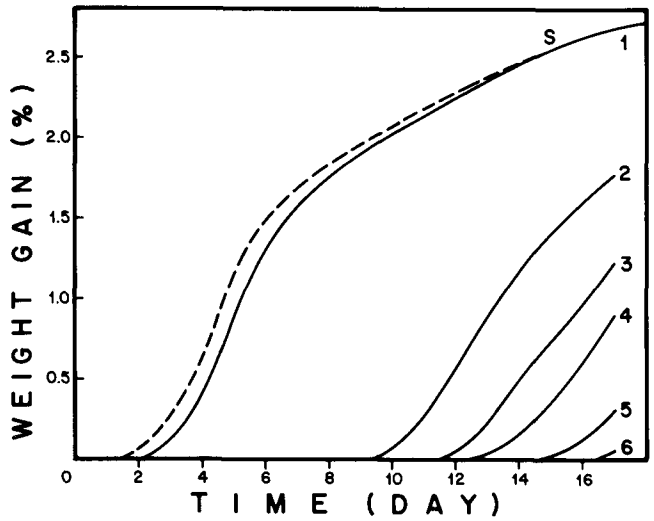

FIG. 2. Comparison of oxidative stability of mackerel skin lipids with various concentrations of TBHQ at 60 C. (S for the control, TBHQ concentrations in the samples are as: 1 – 0.003%, 2 – 0.008%, 3 – 0.012%, 4 – 0.014%, 5 – 0.018%, and 6 – 0.022%). (TBHQ = *tert*-butylhydroquinone.)

and then tested by oven storage for 8 days, have been briefly condensed in Table II. From another viewpoint, the GLC data in Table II also show that alkenals in the sample with 0.02% BHA added, increased twice as fast at the model control oil, oxidized for 8 days under the same reaction conditions. It should be noted that the formation of carbonyls was nearly completely inhibited in the sample treated with 0.02% TBHQ. The reason is that after 8 days of reaction this system was still within its induction period (Fig. 2). In addition to the inhibiting effects of TBHQ on lipid oxidation, it seems to have also slowed down the reaction of lipid hydrolysis by more than 80%; FFA was reduced from 2.9% (the control) to 0.5%. This evaluation does not distinquish between original carboxyl groups and these formed as acids or peracids by autoxidation. The changes of fatty acid composition are not shown in detail but agree with earlier results (13,28) indicating that autoxidation itself, and the antioxidation effects of BHA and TBHQ are nonselective for any component fatty acids.

TBHQ can be used satisfactorily for the effective control of oxidative rancidity for most oxygen-sensitive fat systems. Several key properties of TBHQ make it attractive as an antioxidant for marine lipids. These include no tendency to discolor, no discernible flavor (8,11), excellent solubility in various oils (8,10), and safety in use (12). The trend in recent years towards the inclusion of more highly unsaturated lipids in the diet has led to some encouragement to consume more polyunsaturated marine lipids in the form of various food products as well as fatty fishes. It appears this trend will continue, and TBHQ should fill the need for processed marine oils, fillets from fatty fish (29), as well as the newer processed fishery products based on minced or comminuted fish (30).

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