Investigation of Antioxidants for Polyunsaturated Edible Oils[1]

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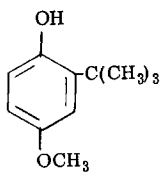
Abstract

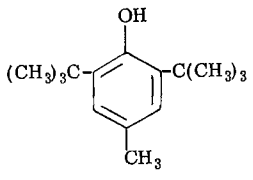
The recent trend toward increased use of polyunsaturated vegetable oils in the human diet has emphasized the need for better antioxidant systems than those currently available. This need led to a research program in which a variety of experimental antioxidants were evaluated. Their selection was influenced by general requirements for food additives and by the results of prior antioxidant studies in various fields. Emphasis was placed on hydroxybenzene types, particularly substituted hydroquinones. Oxidative stability tests employing the standard AOM procedure and 110F shelf storage were used to screen the antioxidants in polyunsaturated oils. The type and number of substituent groups on hydroquinone had considerable effect on antioxidant potency. Some of the experimental compounds, such as 4,4′-methylenebis(5-acenaphthenol) and monoalkylhydroquinones, were several times as effective in the test oils as food-approved antioxidants currently available.

Introduction

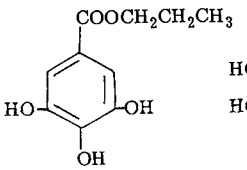
FOR NEARLY 20 YEARS synthetic antioxidants have played an important part in preserving the quality of a variety of edible fats, oils, and food products (1). The most widely used, food-approved stabilizers include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and nordihydroguaiaretic acid (NDGA). Their structures are as follows:



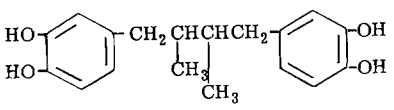
BHA



BHT



PG



NDGA

These "primary" antioxidants are used frequently in mixture with a synergist or a metal deactivator such as citric acid. Government regulations controlling the use of these direct additives in food were outlined in a recent paper (2). Lundberg (3) has tabulated both synthetic and natural food-approved stabilizers employed throughout the world.

[1] Presented at the AOCS Meeting, Cincinnati, October, 1965.

Despite the widespread use of antioxidants, it appears that improvements in food antioxidants are needed. For example, the use of antioxidants in polyunsaturated vegetable oils, such as safflower oil, is restricted due to the relatively low stabilizing value of antioxidants in these oils. Furthermore, other deficiencies of individual antioxidants are indicated, such as the odor of BHT and BHA, the lack of "carry through" of PG and NDGA in food products, the bitterness of NDGA, and the coloration of PG and NDGA due to their contact with iron under some conditions of fat storage and food preparation. Finally, more effective antioxidants which permit adequate stabilization at lower concentrations are desirable.

These considerations led to a screening program aimed at the development of more versatile antioxidants to meet current needs in a broad range of fat, oil, and food products. This paper discloses the results of accelerated oxidation and shelf storage tests on about 60 experimental and food-approved antioxidants in safflower oil. The most effective compounds were tested also in cottonseed oil. A broad variety of types of antioxidants, about 500 compounds, were evaluated during this program. The selection of these compounds was influenced by general requirements for food additives, as well as by the results of prior studies of antioxidants in such substrates as foods, petroleum products, and polymers. However, because of excellent potency as antioxidants in other applications, a number of experimental compounds which possessed possible defects were included purposely. For example, some polyhydroxybenzene derivatives form undesirable colored complexes with metals, some sulfur compounds present odor problems, and some nitrogen compounds are toxic.

The literature discloses numerous references to a variety of antioxidants which have been proposed for fats and oils. Most of these articles have been summarized by Piskur and others in annual literature surveys (4,5). Examples of the diversity of these antioxidants, which include both natural and synthetic products, are shown in Table I. Other aspects of antioxidant technology are pertinent. For example, Miller and Quackenbush discussed the effect of electron-repelling and electron-attracting substituents on the potency of phenolic antioxidants (6). Kim and Kummerow found that both potency and physical

TABLE I

Examples from the Literature of Antioxidants Proposed for Fats and Oils

1.	Derivatives of diphenylamine and phenylenediamines (18)
2.	Biphenyltetrol derivatives (19)
3.	Tetraethylthiuram disulfide (20)
4.	Hydroxy chromans (21)
5.	1-(3,4-Dihydroxyphenyl)-2-propen-1-one derivatives (22)
6.	β-Alkylmercaptoketones (23)
7.	6-Ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (24)
8.	2′,4′,5′-Trihydroxybutyrophenone and homologs (25)
9.	Gentisic acid and sodium gentisate (26)
10.	1-Phenyl-3,5-pyrazolidinedione (27)
11.	Thiobisphenols and methylenebisphenols (28)
12.	Osage orange extract (29)
13.	Quercetin derivatives (30)
14.	Tocopherols (31)
15.	Conidendrols (32)

properties of BHT in corn oil were affected by various substituents on the ring-methyl group (7). Beal et al. studied the stability of safflower oil and its response to antioxidants (8). The effects of light and metals on the stability of certain edible oils have been described (9,10). In a recent extensive review on the polymerization of drying oils, Wexler discussed the mechanisms of oxidation and its inhibition by antioxidants (11). Finally, Lundberg has surveyed the general utility of antioxidants in a wide variety of products (12).

Experiments and Results

Two different oils were used in this work. These were commercial lots of refined safflower and cottonseed oils, which contained no added antioxidant. Both had zero peroxide content as received, and were kept refrigerated in glass containers until used. They were selected because of the recent interest in use of polyunsaturated fats in the human diet.

All of the antioxidants were screened at 0.05 wt % concentration in the safflower oil by two different oxidative stability tests. One of these was the well-known active oxygen method (AOM) of Riemenschneider (13), wherein air is bubbled through a 25-ml oil sample at 210F. The other test consists of storing 25-ml oil samples at 110F in loosely capped 4-oz amber glass bottles in a dark, forced-air oven. In each test, oil stability consists of determining the time required to develop a peroxide number of 70 (meq peroxide/kg oil) by the Wheeler iodometric procedure (14). The use of two different oxidation tests provided information on the effects of temperature and dynamic aeration on antioxidant potency.

Following the screening phase, selected antioxidants were tested in both oils at lower concentrations which are more in line with current practice.

Discussion

While food-grade antioxidants must meet several varied requirements, it is obvious that first they must be effective in retarding oxidation. Thus, potency was selected as the basis for the screening program. Due to the number of compounds evaluated in the program, only single tests were conducted during the screening phase. However, the results of duplicate tests by both the AOM and the 110F storage procedures are shown for several compounds in Table II and indicate reasonably good repeatability.

TABLE II

Potency of Commercial Antioxidants in Safflower Oil

Entry	Antioxidant 0.05 wt % in oil	Oil life (time to peroxide no. 70)[a]	
		AOM at 210F hr	Storage at 110F days
1	None (control)	9	21
	Food-approved antioxidants		
2	Butylated hydroxyanisole (BHA)	9;10[b]	21;21[b]
3	Butylated hydroxytoluene (BHT)	13;12	49;50
4	Propyl gallate (PG)	24;23	103;81
5	Nordihydroguaiaretic acid (NDGA)	12	56
6	2′,4′,5′-Trihydroxybutyrophenone	21;18	90;101
	Other commercial antioxidants		
7	3,3′-Thiodipropionic acid	65	193
8	Dilauryl 3,3′-Thiodipropionate (at 0.072%)	9	113
9	4,4′-Thiobis[6-*tert.*-butyl-m-cresol]	8	19
10	2,2′-Thiobis[6-*tert.*-butyl-p-cresol]	10	21
11	6-Ethoxy-1,2-dihydro-2,2,4-trimethylquinoline	11	14
12	N,N′-Diphenyl-p-phenylenediamine	11	

[a] Meq peroxide/kg oil.
[b] Duplicate tests.

The effect of oxidation test temperature on antioxidant potency was of interest. For most of the compounds included in this study, antioxidant ratings did not vary apreciably between the AOM test and the 110F storage procedure. In other words, a compound rated "good" by the AOM test was also "good" in the 110F storage phase. It is interesting that in the absence of antioxidants the stability, or life, of the safflower oil was 9 hr by the AOM test and 21 days by the 110F storage procedure.

Food-Approved Antioxidants

The food-approved antioxidants had moderate-to-low potency in safflower oil (Table II). The most effective of them, PG, increased the AOM life to 24 hr and the 110F storage life to about 92 days. PG was followed in decreasing order of activity by 2′,4′,5′-trihydroxybutyrophenone (THBP) (15), BHT and NDGA, and BHA.

Other Commercial Antioxidants

Several other commercial antioxidants, some of which are not approved for direct addition to foods, are listed in Table II also. They include well-known sulfur and nitrogen derivatives which are said to be effective antioxidants in products other than foods. With the exception of 3,3′-thiodipropionic acid (TDPA), these antioxidants had little activity. Despite food approved for some applications, apparently TDPA is not used appreciably in edible products due to odor and solubility problems (1).

Hydroquinone and Catechol Derivatives

Several hydroquinone derivatives were found to be potent antioxidants in polyunsaturated vegetable oil (Table III). In fact, the activity of hydroquinone itself (39-hr AOM life) was increased to about 80 hr by the presence of certain substituent groups. This enhancement would have been even more pronounced if the comparison had been made on a molar basis rather than a weight basis. With one alkyl group on the benzene ring, the highest potency was obtained with the butyl group (about 80 hr). In regard to the alkyl group configuration, little difference in potency was observed between the *sec.*-butyl and the *tert.*-butyl groups by the AOM test, but a highly branched octyl group was superior to

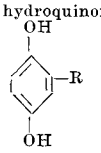
TABLE III

Potency of Hydroquinone and Catechol Derivatives in Safflower Oil

Entry	Antioxidant 0.05 wt % in oil	Oil life (time to peroxide no. 70)[a]	
		AOM at 210F hr	Storage at 110F days
1	None (control)	9	21
2	Hydroquinone	39	191
3	Methylhydroquinone	69	330
4	2,3-Dimethylhydroquinone	36	261
5	2,5-Dimethylhydroquinone	36	161
6	2,3,5-Trimethylhydroquinone	19	65
7	Tetramethylhydroquinone	9	9
8	Isopropylhydroquinone	68	320
9	*sec.*-Butylhydroquinone	80	256
10	*tert.*-Butylhydroquinone	79	372
11	2,5-Di-tert-butylhydroquinone	42	263
12	Pentylhydroquinone	50	203
13	Cyclohexylhydroquinone	49	175
14	Octylhydroquinone	46	208
15	(1,1,3,3-Tetramethylbutyl)hydroquinone	61	253
16	Dodecylhydroquinone	34	162
17	Methoxyhydroquinone	61	296
18	Chlorohydroquinone	22	80
19	3,6-Dihydroxyphthalic acid	10	44
20	Diethyl 3,6-dihydroxyphthalate	9	25
21	Catechol	12	25
22	4-*tert.*-Butylcatechol	12	38
23	3-Methyl-6-isopropylcatechol	13	69

[a] Meq peroxide/kg oil.

TABLE IV
Effect of Substituents on Potency of Hydroquinone in Safflower Oil

Entry	Antioxidant 0.05 wt % in oil	R on hydroquinone 	Oil life (time to peroxide no. 70)[a] AOM at 210F hr	Oil life (time to peroxide no. 70)[a] Storage at 110F days
1	None	.....	9	21
2	Hydroquinone	—H	39	191
3	2,5-Dihydroxyphenyl methyl ketone	—$COCH_3$	9	22
4	2′,5′-Dihydroxybutyrophenone	—$COCH_2CH_2CH_3$	9	26
5	(1,1,3,3-Tetramethylbutyl)-hydroquinone	—$CH_2C(CH_3)_2CH_2C(CH_3)_3$	61	253
6	Phenylhydroquinone	—C_6H_5	37	169
7	Methoxyhydroquinone	—OCH_3	61	296
8	Phenoxyhydroquinone	—OC_6H_5	30	112
9	Gentisic acid	—COOH	14	37
10	Propyl gentisate	—$COOCH_2CH_2CH_3$	9	21

[a] Meq peroxide/kg oil.

a normal octyl group. In regard to the effect of the number of alkyl groups on the benzene ring, the data for five different methyl-substituted hydroquinones provide an interesting comparison (entries 3–7, Table III). While the presence of one methyl group caused an increase in potency, the presence of two, three, or four methyl groups caused a progressive decrease in potency to the point of complete inactivity for tetramethylhydroquinone. The superiority of one alkyl substituent over two is seen also in the case of the *tert.*-butyl derivatives (entries 10 and 11). A methoxy group appeared to be a desirable substituent on hydroquinone (entry 17), but a chloro group reduced the potency (entry 18).

In contrast to the hydroquinone series, catechol and two of its alkylated derivatives had low antioxidant potency in safflower oil.

Additional information on the effect of substituents on the potency of hydroquinone is shown in Table IV. The following groups caused reduction in antioxidant value: ketone (entries 3 and 4), carboxy (entry 9), and ester (entry 10). The octyl group was superior to the phenyl group (entries 5 and 6), and the methoxy group was preferable to the phenoxy group (entries 7 and 8). It should be emphasized that these group effects, as well as the relative ratings assigned to the different antioxidant types, may not apply in other substrates, such as gasoline or rubber.

Other Hydroxy-Aromatic Compounds

A series of alkylated phenols, with variation in number, location, and configuration of the alkyl groups, were relatively ineffective as antioxidants in safflower oil (Table V). Some of these compounds were similar to BHT in potency.

In the benzenetriol series, 1,2,4-benzenetriol was very effective (89-hr AOM life), being more potent than 1,2,3-benzenetriol.

In the naphthol series, 1,5-naphthalenediol and 1,7-naphthalenediol were much more effective than 1-naphthol. Certain naphthalenediols presumably can lose protons, while functioning as antioxidants, to assume quinoidal structures in the manner proposed for hydroquinone and catechol, as follows:

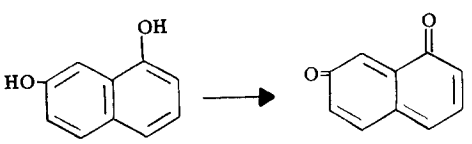
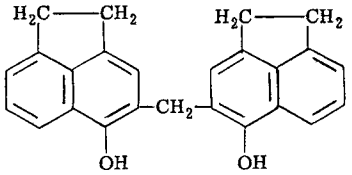


TABLE V
Potency of Miscellaneous Hydroxy Aromatic Compounds in Safflower Oil

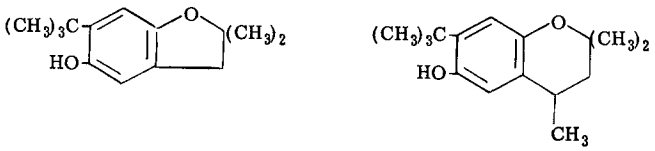
Entry	Antioxidant 0.05 wt % in oil	Oil life (time to peroxide no. 70)[a] AOM at 210F hr	Oil life (time to peroxide no. 70)[a] Storage at 110F days
1	2,6-Di-*tert.*-butylphenol	9	28
2	2,6-Di-*tert.*-butyl-4-ethylphenol	10	36
3	2,6-Di-*tert*-butyl-4-isopropylphenol	11	31
4	2,4,6-Trimethylphenol	11	29
5	6-*tert.*-Butyl-2,4-xylenol	11	33
6	2,4,6-Tris(1-methylheptyl)phenol	9	
7	1,2,3-Benzenetriol	28	177
8	4-Butyl-1,2,3-benzenetriol	28	165
9	1,2,4-Benzenetriol	89	511
10	5-*tert.*-Butyl-1,2,4-benzenetriol	30	145
11	Gallic acid	27	88
12	Hexyl gallate	22	89
13	1-Naphthol	15	31
14	1,5-Naphthalenediol	25	100
15	1,7-Naphthalenediol	26	80
16	5-Acenaphthenol	92	320
17	4,4′-Methylenebis(5-acenaphthenol)	115	544
18	6-*tert.*-Butyl-2,2-dimethyl-5-benzofuranol	16	29
19	7-*tert.*-Butyl-2,2,4-trimethyl-6-chromanol	11	27

[a] Meq peroxide/kg oil.

Among the most potent antioxidants found in this work were 5-acenaphthenol and 4.4′-methylenebis(5-acenaphthenol), as seen in entries 16 and 17 of Table V (16). The latter compound, which has the following structure, increased the AOM life of the safflower oil to 115 hr.

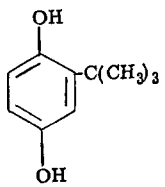


Certain chromanols and benzofuranols, which have structural components similar to those of BHA, have been proposed as antioxidants (17). However, in safflower oil, compounds of these types (entries 18 and 19, Table V) were relatively inactive. Their structures are:



Extensive Evaluation

On the basis of potency, solubility, and other considerations, *tert.*-butylhydroquinone (TBHQ) was selected for extensive comparison with food-approved antioxidants as a potential stabilizer for edible fats and oils. Its structure is as follows:



The results of both the AOM test and the 110F storage tests at several concentrations of antioxidant in safflower and cottonseed oils are shown in Figures 1 and 2. *tert.*-Butylhydroquinone was quite superior to the food-approved antioxidants in both oils at concentrations from 0.01 to 0.05%. At a concentration of 0.025%, *tert.*-butylhydroquinone was between two and three times as effective as propyl gallate in increasing oil life in both oxidation tests.

In summary, the need for more useful antioxidants in polyunsaturated edible oils led to a screening pro-

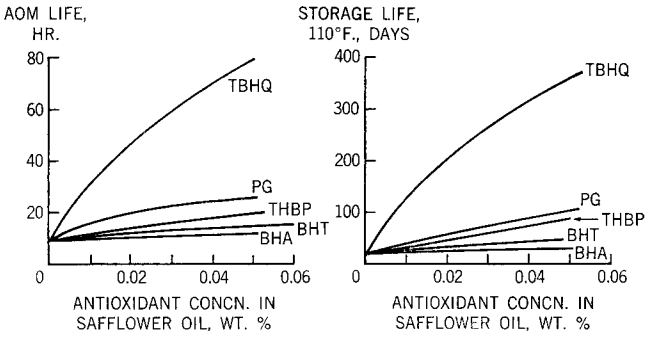


FIG. 1. Antioxidant potency in safflower oil.

gram which disclosed several experimental stabilizers with higher potency than current food-approved antioxidants. Good correlation was observed between the 210F AOM and the 110F storage ratings for most of the antioxidants studied. On the basis of encouraging preliminary results and toxicity studies, one of these compounds, *tert.*-butylhydroquinone, is undergoing further evaluation for potential use as a food-grade antioxidant.

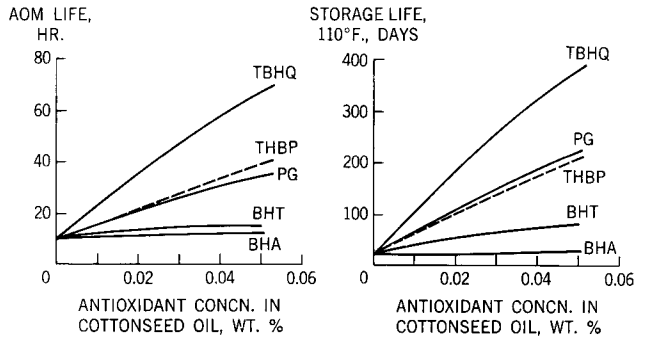


FIG. 2. Antioxidant potency in cottonseed oil.

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