Butylated Hydroxyanisole as an Antioxidant for Animal Fats[1]

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IN recent years the consumption of products of the commercial bakery has increased greatly. Many baked products, such as crackers and cookies, are now packaged and held as shelf items in the grocery stores for considerable periods of time. To meet these conditions adequately, especially in warm weather, it is necessary that an antioxidant be added to animal fats that will carry through and protect from rancidity the foods made with the fats.

Remarkable progress has been made in recent years in developing antioxidants which protect the fat from rancidity. This progress has been reviewed in a recent survey by Lundberg (1). Permission for the use of a number of these antioxidants in animal fats and shortenings containing animal fats has been given by the Federal Meat Inspection Division (2). Although some of these antioxidants give adequate protection to the fat, they do not carry through into the foods to give effective protection against rancidity.

For a number of years our laboratory has been testing many compounds in an effort to find an antioxidant that would more nearly meet the requirements of an ideal antioxidant. Higgins and Black (3) have stated the requirements of an ideal antioxidant. These requirements are:

1. It should exert no harmful physiological effect.
2. It should contribute no objectionable flavor, odor, or color to the fat or the foods made with the fat.
3. It should carry through and effectively protect the foods made with the fat from rancidity.
4. It should be sufficiently fat soluble so that it can be added to the fat with ease.
5. It should be effective in low concentrations.
6. It should be readily available in adequate amounts.
7. It should be reasonable in cost.

We have found that butylated hydroxyanisole and certain mixtures of compounds containing it more nearly meet the requirements of an ideal antioxidant than any other antioxidant we have studied.

Solubility of Butylated Hydroxyanisole

Butylated hydroxyanisole (BHA) consists chiefly of two isomers: 3-tertiarybutyl-4-hydroxyanisole (2-tertiarybutyl-4-methoxyphenol) and 2-tertiarybutyl-4-hydroxyanisole. It is readily soluble in fats in all properties, but nearly insoluble in water.

The following procedure was used to determine the solubility of BHA in water. Duplicate samples of BHA (0.1 g.) and water (10 ml.) were shaken in a constant temperature bath at 25°C. One-ml. aliquots were withdrawn from each flask after 3 and 22 hours respectively. The concentration of BHA was determined colorimetrically by an adaptation of the Emmerie and Engel method for tocopherols (4). The temperature was raised to 70°C. and one-ml. aliquots withdrawn and tested as before. It was found that BHA is soluble in water to the extent of 0.00154 g./100 ml. at 25°C. and 0.00165 g./100 ml. at 70°C.

TABLE I

Solubility of BHA in Water and Its Extractability From Lard

Shaking Time (Hours)	Temp.°C.	Original Condition of BHA	% Concentration of BHA in Water
3	25.5	Solid	0.00153
3	25.6	Melted	0.00164
22	24.7	Solid	0.00151
22	24.7	Melted	0.00148
			0.00154 Avg.
2	70.0	Melted	0.00159
2	70.0	Melted	0.00172
			0.00165 Avg.
18	70.0	In Lard	0.00025
18	70.0	In Lard	0.00024
22	70.0	In Lard	0.00000
22	70.0	In Lard	0.00000

To determine the extractability of BHA from lard by water, duplicate samples of 5 g. of lard containing 0.015% of BHA and 10 ml. of water were shaken at 70°C. One-ml. aliquots of the aqueous phase were withdrawn after 18 and 22 hours, and the concentration of BHA determined. Good separation of the aqueous from the lard phase was not obtained in the samples withdrawn at 18 hours. Water extraction at 70°C. failed to remove a measurable amount of BHA from the lard (Table I).

Antioxidant Activity of BHA Alone

When used alone, BHA is not unusually effective in increasing the stability of lard as measured by the Active Oxygen Method (5) (Tables II and III). A four-hour lard with 0.005% of BHA is usually increased to about 16 to 18 hours, and with 0.01% to

TABLE II

Average Stability of Lard by Active Oxygen Method

Treatment		Hours Stability							
Control	Per cent	½	1½	2	3	4	5	6	8
BHA*	0.005	....	....	....	14 (1)	16 (3)	23 (4)	....	....
3 isomer**	0.005	....	....	12 (1)	13 (3)	19 (5)	22 (3)	....	....
BHA	0.01	4 (1)	16 (1)	17 (7)	21 (4)	21 (1)	34 (1)	31 (4)	32 (3)
3 isomer	0.01	....	....	20 (2)	23 (1)	28 (4)	32 (5)	34	....

* Butylated hydroxyanisole.
** 3-tertiarybutyl-4-hydroxyanisole.
Numbers in parentheses indicate number of tests.

TABLE III

Relation of Concentration of BHA to Stabilizing Effect Expressed in AOM Hours

Stability of Lard Without Antioxidant 4 Hours

Per cent antioxidant	0.005	0.01	0.02	0.04	0.06	0.08	0.10
Hours AOM	18	25	29	28	30	23	24

[1] Presented at the meeting of the American Oil Chemists' Society, Nov. 17, 1948, New York City.

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about 20 to 25 hours. The increase in stability is generally slightly higher in the lard of the higher initial stability. Increasing the concentration of BHA from 0.005% to 0.02% results in increased stability, but further increases in amounts up to 0.10% do not seem to increase the stability (Table III). There is evidence that increasing the amounts beyond 0.06% results in decreased stability. A similar effect was noted by Swift, Rose, and Jamieson (6) with tocopherol. Lundberg, Dockstader, and Halvorson (7) noted the same effect with NDGA and other phenols. They pointed out that the positive catalytic effect on the formation of peroxides during the initial stages of autoxidation seems to be characteristic of phenols in general; and that, because of this phenomenon, it is found that each such phenolic antioxidant possesses an optimal concentration insofar as the prevention of organoleptically detectable rancidity is concerned.

Effect of Synergists with BHA

BHA exhibits synergism with citric acid, phosphoric acid, triethyl phosphate, ethyl acid phosphate, and lecithin (Table IV). In general, these synergists increase the AOM values from 5 to 8 hours. The extent of the effect of acid synergists varies with different lots of lard.

Methionine in 0.01% concentration exhibits synergism with BHA similar to the effect obtained with 0.002% of the acid type synergists (Table IV). The effect is similar to that observed by Clausen, Lundberg, and Burr (8) with certain phenolic antioxidants.

Synergism of BHA, Hydroquinone and Phosphoric Acid

The effect of BHA in combination with hydroquinone and phosphoric acid on the stability of lard is shown in Table V. In the first case, with a three-hour lard, BHA in 0.01% and hydroquinone in 0.003% each increased the AOM value 18 hours. When they were used in combination, the AOM value was increased 42 hours. Thus, there was a mutual synergistic effect of 6 hours between the hydroquinone and the BHA. Golumbic (9) observed mutual synergism between certain pairs of phenolic antioxidants having different oxidation-reduction potentials. But Lundberg, Halvorson, and Burr (10) found that NDGA and the tocopherols do not exhibit mutual synergism. When phosphoric acid in 0.002% was used with the BHA and hydroquinone, the stability was increased to 47 hours. The additional synergistic effect due to the phosphoric acid was 5 hours. Similar values are shown for the second lard.

Synergism of BHA, Thiodipropionic Acid and Phosphoric Acid

Combinations of BHA and thiodipropionic acid are extremely effective in increasing the stability of lard as measured by the active oxygen method (Table V). The effectiveness increases sharply at values of 0.005 to 0.01% of thiodipropionic acid. The effectiveness of thiodipropionic acid alone increases greatly with increase in concentration. This is in sharp contrast to the effect of BHA when used alone. The combination of thiodipropionic acid and BHA exhibits unusual synergism. The effectiveness of thiodipropionic acid is reduced by the addition of 0.005% of phosphoric acid in one case and not in the other. In both lards the effectiveness of the combination of BHA and thiodipropionic acid is reduced by the addition of 0.005% of phosphoric acid.

Synergism of BHA, Propyl Gallate, Hydroquinone and Acids

Combinations of BHA with propyl gallate exhibit synergism with citric acid and ethyl acid phosphate similarly to combinations of BHA and hydroquinone (Table VI). With a four-hour lard, the synergistic effect with BHA 0.01%, propyl gallate 0.003%, and ethyl acid phosphate 0.002% was 15 hours, and with citric acid replacing the ethyl acid phosphate 17 hours. With BHA 0.01%, hydroquinone 0.003%, and citric acid 0.002%, the synergistic effect was 15 hours. No particular significance is attached to the difference in synergistic effects of ethyl acid phosphate and citric acid.

TABLE IV

Effect of Synergists With BHA

Antioxidant	Per cent	AOM	Difference from control	Synergism
		Hrs.	*Hrs.*	*Hrs.*
None	.....	4	....	....
BHA	0.005	16	12	....
BHA H_3PO_4	0.005 0.005	22	18	6
None		2	....	....
BHA	0.01	16	14	....
BHA H_3PO_4	0.01 0.002	21	19	5
BHA Tri ethyl phosphate	0.01 0.002	20	18	4
BHA Ethyl acid phosphate	0.01 0.002	21	19	5
None	.....	2	....	....
BHA	0.01	16	14	....
BHA Citric acid	0.01 0.002	36	34	20
None	.....	8	....	....
BHA	0.01	32	24	....
BHA Citric acid	0.01 0.005	39	31	7
None	.....	5	....	....
BHA	0.005	17	12	....
BHA Citric acid	0.005 0.01	40	35	18
None	.....	2	0	....
BHA	0.01	16	14	....
Lecithin	0.10	4	2	....
BHA Lecithin	0.01 0.10	26	24	8
BHA Lecithin	0.01 0.01	21	19	3 to 5
None	.....	8	0	....
BHA	0.01	32	24	....
Methionine	0.01	10	2	....
BHA Methionine	0.01 0.01	38	30	4
BHA Citric acid	0.01 0.005	39	31	7
Methionine Citric acid	0.01 0.005	10	2	0
BHA Methionine Citric acid	0.01 0.01 0.005	46	38	12

TABLE V

Synergism of BHA, Hydroquinone, Thiodipropionic Acid, and Phosphoric Acid

Antioxidant	Per cent	AOM	Difference from control	Synergism BHA and Hq.	Synergistic effect of H_3PO_4	Total synergism
		Hrs.	*Hrs.*	*Hrs.*	*Hrs.*	*Hrs.*
None	.....	3	0			
BHA	0.01	21	18			
Hq.	0.003	21	18			
H_3PO_4	0.002	3	0			
BHA Hq.	0.01 0.003	45	42	6		6
BHA Hq. H_3PO_4	0.01 0.003 0.002	50	47		5	11
None	.....	2	0			
BHA	0.01	21	19			
Hq.	0.003	22	20			
H_3PO_4	0.002	2	0			
BHA Hq.	0.01 0.003	45	43	4		4
BHA Hq. H_3PO_4	0.01 0.003 0.002	51	49		6	10

Antioxidant	Per cent	AOM	Difference from control	Synergism BHA and TDPA	Synergistic effect of H_3PO_4	Total Synergistic effect
		Hrs.	*Hrs.*	*Hrs.*	*Hrs.*	*Hrs.*
None	.....	2	0			
H_3PO_4	0.005	2	0			
BHA	0.01	38	36			
TDPA	0.01	19	17			
BHA TDPA	0.01 0.01	81	79	26		26
TDPA H_3PO_4	0.01 0.005	4	2		—15	—15
BHA TDPA H_3PO_4	0.01 0.01 0.005	67	65		—14	12
BHA H_3PO_4	0.01 0.005	45	43		7	7
None	.....	7	0			
H_3PO_4	0.005	7	0			
BHA	0.01	40	33			
TDPA	0.01	50	43			
BHA H_3PO_4	0.01 0.005	53	46		13	13
TDPA H_3PO_4	0.01 0.005	55	48		5(?)	5(?)
BHA TDPA	0.01 0.01	145	138	62		62
BHA TDPA H_3PO_4	0.01 0.01 0.005	117	110		—28	34

Effect of BHA on Shelf Life of Lard

Shelf life storage tests were made by placing 20 grams of melted lard in clean 4-ounce glass sample jars and covering the jars with loosely fitted screw top lids so that there was access of air. The jars were placed at room temperature in the dark and examined organoleptically at monthly intervals for rancidity. When rancidity was detected, the end point was checked chemically by determining the peroxide value. BHA and combinations with synergists have proved to be very effective in prolonging the keeping time of lard (Table VII). The lards without antioxidants became rancid at the end of 3 and 5 months. Those stabilized with BHA were kept free from rancidity for from 23 to 32 months.

TABLE VI

Synergism of BHA, Propyl Gallate, Hydroquinone, and Acids

Antioxidant	Per cent	AOM	Difference from control	Synergism
		Hrs.	*Hrs.*	*Hrs.*
None	.....	4	....	....
BHA	0.01	21	17	....
EAP	0.002	5	1	....
PG	0.003	26	22	....
Hq	0.003	27	23	....
BHA PG EAP	0.01 0.003 0.002	59	55	15
BHA PG CA	0.01 0.003 0.002	60	56	17
BHA Hq CA	0.01 0.003 0.002	59	55	15

EAP = ethyl acid phosphate. PG = propyl gallate. Hq. = hydroquinone. CA = citric acid.

Effectiveness of BHA, Hydroquinone and Citric Acid

Animal fat antioxidants consisting of a combination of BHA with hydroquinone and citric acid designated as AMI 72 (11) and a modification in which hydroquinone is replaced by propyl gallate, AMIF 72 (14), were found to be very effective and to offer much promise. The concentrations of the ingredients of AMI 72 as used in this paper are as follows:

AMI 72 0.015%: BHA 0.01%, Hq. 0.003%, and CA 0.002%.
AMI 72 0.025%: BHA 0.02%, Hq. 0.003%, and CA 0.002%.
AMI 72 0.017%: BHA 0.01%, Hq. 0.005%, and CA 0.002%.

A large number of pilot plant tests have shown that the antioxidant is not only effective in increasing the stability of lard as measured by the AOM value, but that it is very effective in increasing the shelf life of the lard. Furthermore many tests have shown the superiority of this antioxidant in protecting the foods made with lard from rancidity.

TABLE VII

Shelf Storage Tests on Lard

Antioxidant	Per cent	Shelf Life (months)	Peroxide Value (avg.)
None	.....	3	All rancid
BHA	0.01	24	18
BHA H_3PO_4	0.01 0.005	24	24
BHA	0.02	24	12
BHA H_3PO_4	0.02 0.005	24	19
None	.....	5	All rancid
BHA H_3PO_4	0.01 0.005	29	24
None	.....	5	....
BHA	0.005	26 31	30 non-rancid odor All rancid
BHA H_3PO_4	0.005 0.01	26 33	28 non-rancid odor 65 rancid
BHA	0.01	26 33	20 non-rancid odor 29 rancid
BHA H_3PO_4	0.01 0.01	31 33	24 non-rancid odor 21 rancid
H_3PO_4	0.01	7 9	11 non-rancid 75 all rancid

Effect of Antioxidants in Foods Made With Lard

The effect of different antioxidants in protecting the foods made from lard is shown in Table VIII. The following is a description of the samples of lard used in the tests:

Lard No. 1 was a steam-rendered lard, to which about 10% of lard flakes were added. Approximately 1,000-pound batches were treated with the respective antioxidants and then plasticized.

Lard No. 4, a steam-rendered lard, was caustic soda refined. A 4,500-pound batch, to which lard flakes had been added, was deodorized. One-hundred-pound batches of the deodorized lard were treated with the respective antioxidants.

Lard No. 7, a steam-rendered lard, was bleached and deodorized. Lard flakes and mono- and di-glycerides were added. Approximately 800-pound batches of lard were treated with the respective antioxidants.

Lard numbers 8, 10, and 11, all steam-rendered lards, were treated with antioxidants in the laboratory.

The extent to which antioxidants carry through and protect from rancidity the foods made with lard was determined in three products: pastry, potato chips, and crackers.

Pastry (pie crust) was made from the fat being studied, using 44% as much fat as flour and was baked 16 minutes at 425°F. After baking and cooling, two wafers were stored in each of four screw top glass jars (4-oz. mayonnaise jars), in an oven at 145°F. until rancidity developed. All the samples held at 145°F. were smelled daily for rancidity.

To study the effect of frying conditions, five 100-gram lots of potato slices were fried in 1,300 grams of the fat heated to 375°F., during a 30-minute cooking period. The fourth and fifth lots were combined, crumbled, mixed thoroughly, and portions stored in four glass jars in an oven as for pastry wafers.

The third product tested was crackers. These were made in accordance with the procedure used by the Technical Institute of the Independent Biscuit Manufacturers Company, inc., Chicago. In brief, it consists in making soda crackers from a typical formula, using 100 grams of flour, baking 9 minutes at 480°F., crisping the following day, and storing at 145°F. in glass jars.

It will be noted that the antioxidant AMI 72 is superior in protecting the stability of potato chips, pastry, and crackers. NDGA, propyl gallate, lauryl gallate, and hydroquinone showed a relatively small increase in the stability of potato chips and pastry. AMI 72 was the only antioxidant to show any significant effect in crackers.

The propyl gallate and lauryl gallate were added in quantities molecularly equivalent to each other. The results on AOM stability were very comparable, confirming the results obtained by Morris *et al.* (12).

Higgins and Black (3) obtained good protection in crackers with 0.1% gum guaiac in lard and some protection with 0.01 per cent NDGA. Tocopherols and propyl gallate in 0.01% concentration failed to increase the stability of crackers. Lundberg, Halvorson, and Burr (10) found some increase in stability of pastry and crackers with NDGA when used in 0.1% in the lard. However, this concentration is 10 times the amount permitted by the regulation of the Federal Meat Inspection Division. Stevens and Thompson (13) found no significant increase in stability of army ration biscuits when 0.02% NDGA was used in the lard.

In lard No. 10 two similar antioxidant mixtures containing propyl gallate were used, differing only in the method by which they were added to the lard. The first mixture was blended by heating and stirring until clear and then stirring until the mixture cooled. The mixture was then added to lard. The second mixture was not blended, but each ingredient was added separately in ethanol solution to the lard.

TABLE VIII

Effectiveness of Antioxidants in Protecting Foods Made With Lard From Rancidity

Antioxidant	Per cent	Stability AOM	Potato Chips* Stability Schaal Oven	Pastry Stability Schaal Oven	Crackers Stability Schaal Oven
		Hrs.	*Hrs. 145°F.*	*Hrs. 145°F.*	*Hrs. 145°F.*
Lard No. 1 none		1	22	28	203
NDGA CA	0.001 0.005	29	72	35	213
AMI 72	0.015	68	705	451	817
AMI 72	0.025	73	1243	979	1040
Lard No. 4 none		3	21	40	199
NDGA CA	0.0015 0.005	27	118	50	212
PG	0.01	18	160	68	224
AMI 72	0.015	72	672	766	570
AMI 72	0.025	80	918	917	609
Lard No. 7 none		1	14	16	139
NDGA H_3PO_4	0.002 0.002	3	58	40	121
AMI 72	0.015	48	761	551	493
AMI 72	0.025	56	1424	904	691
Lard No. 8 none		3	31	19	198
PG	0.01	41	184	43	145
LG	0.016	41	172	79	210
AMI 72	0.025	62	1142	787	648
Lard No. 10 none		10	49	43	289
PG Lecithin Corn oil CA	0.0075 0.0075 0.027 0.002	58	262	86	264
PG Lecithin Corn oil CA	0.0075 0.0075 0.027 0.002	70	292	90	212
AMI 72	0.017	88	954	714	937
Lard No. 11 none		8	50	49	454
Hq. CA	0.01 0.002	100	248	65	394

NDGA = nordihydroguaiaretic acid.
CA = citric acid.
AMI 72 0.015%:
0.01% butylated hydroxyanisole
0.03% hydroquinone
0.002% citric acid.
AMI 72 0.017%:
0.01% butylated hydroxyanisole
0.005% hydroquinone
0.002% citric acid.
AMI 72 0.025%:
0.02% butylated hydroxyanisole
0.003% hydroquinone
0.002% citric acid.
PG = propyl gallate.
LG = lauryl gallate.
Hq. = hydroquinone.
* These are results of laboratory tests. The keeping times are longer than would be expected in commercial production.

In a subsequent experiment similar AOM values were obtained from the two mixes, but in neither case was there any evidence that pre-blending of the ingredients exerted a more favorable effect than the separate addition of the ingredients.

Commercial Baking Test with Crackers

Two 450-pound batches of lard treated with antioxidant and a similar sized batch of lard without antioxidant were shipped to a commercial cracker bakery. Samples of the lots of lard were obtained for laboratory tests. The stability of the commercially baked crackers was determined independently in two laboratories. The results of these tests and of the laboratory baking tests are given in Table IX. It will be noted that there is good agreement between the results of the laboratory and commercial baking tests and in the stability tests of the two laboratories.

TABLE IX

Comparison of Effectiveness of AMI 72 in Laboratory and Commercially Baked Crackers

Antioxidant	Per cent	Stability of lard AOM	Stability Laboratory baked crackers 145°F.	Stability of Commercially Baked Crackers 145°F.			
				Type 1 Laboratory Test		Type 2 Laboratory Test	
				No. 1	No. 2	No. 1	No. 2
		Hrs.	*Hrs.*	*Hrs.*	*Hrs.*	*Hrs.*	*Hrs.*
None	.....	8	138	188	156	239	195
AMI 72	0.015	74	535	606	532	576	564
AMI 72	0.025	84	763	732	786	817	696

We believe that the laboratory tests with pastry and crackers give results comparable to those which may be expected under commercial conditions. The conditions of frying potato chips in the laboratory are quite different from commercial conditions. For that reason the keeping times in the laboratory tests are longer than would be expected under commercial operations. The method does serve however to compare the effectiveness of the different antioxidants.

Toxicological Studies

Extensive studies on the toxicity of BHA have been carried out over a period of three years. The studies with rats were carried out through the entire life cycle and involved reproduction and second generation of animals. No harmful physiological effects were found when the antioxidant was used daily in the food even in amounts several hundred times the maximum amount permitted by the regulations of the Meat Inspection Division. Permission for the use of BHA alone and in combination with other antioxidants and synergists in animal fats and shortenings containing animal fats has been granted by the Federal Meat Inspection Division, Bureau of Animal Industry, U.S.D.A. (Memorandum No. 118, December 1948).

Commercial Use of the Antioxidant

The ingredients of AMIF 72 are easily dissolved in propylene glycol solution to give a convenient preparation for commercial use. One pound or one pint of a solution of 70 parts propylene glycol, 20 parts BHA, 6 parts propyl gallate, and 4 parts of citric acid when added to 2,000 pounds of fat will give a concentration of 0.01% BHA, 0.003% propyl gallate and 0.002% of citric acid in the fat. The antioxidant solution is merely added to the liquid fat (165°-175° F.) and followed by thorough agitation to insure complete distribution of the antioxidant through the fat. It may be added to the fat by means of a proportioning pump. The propylene glycol solution is stable and may be shipped in suitable drums or other containers.

Summary

Butylated hydroxyanisole has been developed as a new and very effective antioxidant for animal fats. It is readily soluble in fats, and practically insoluble in water. It exhibits synergism with acids, hydroquinone, methionine, lecithin, and thiodipropionic acid. It is very effective in protecting foods made with lard against rancidity.

In combination with small quantities of hydroquinone or propyl gallate and an acid synergist, it imparts to animal fats high AOM stability and shelf life and is very effective in protecting foods made with lard (crackers, pastry, etc.) against rancidity.

Extensive pilot plant and commercial tests have demonstrated the practical usefulness of the antioxidant.

Extensive toxicological tests with rats have failed to demonstrate any physiological effects when the antioxidant is used daily in food, even in amounts several hundred times the maximum amount permitted by the regulations of the Meat Inspection Division.

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