The Development of a Practical Antioxidant* for Lard and Shortening

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Introduction

Since it was first shown that corn and wheat-germ oils (1, 11) or their unsaponifiable fractions (8) could be used to improve the resistance to oxidation of less stable fats, various means of utilizing the natural inhibitors present in crude oils and in vegetable tissues have been suggested. A review of the literature suggests the following guiding principles in the choice and application of an efficient and practical antioxidant.

1. With processed foods the antioxidant should have its maximum effect when it is incorporated into the fat. To accomplish this the antioxidant should, preferably, be in oil solution.

2. The base oil itself should have antioxidant properties.

3. A mixture of the two common types of antioxidants, acidic and phenolic, should be employed since it is more active than either one alone (3).

4. Apart from any synergistic effect, both kinds of stabilizers should be present in an antioxidant which is intended for general use, since the acidic type is more effective in vegetable fats and oils than in those of animal origin, while the reverse is true of the phenolic type (9).

5. The antioxidants and their carrier must be natural occurring non-toxic substances acceptable to the Food and Drugs Administration and without deleterious effect on the characteristic flavor, natural texture and color of the food.

The researches described herein are concerned with the development of an antioxidant according to these specifications and which is essentially wheat-germ oil fortified with citric acid.

Experimental

METHODS OF ANALYSIS:

I. *Aeration.* In order to provide a ready means of comparison with results obtained in industrial laboratories, an adaptation of the Swift Stability Test (6) was used for the majority of the estimations of antioxidant activity. The Swift Test gives the relative stability to the nearest hour, but in this laboratory, a refinement of the Hubata indicator method (4) yielded results which could be closely reproduced, duplicate samples checking within two percent of the mean induction time. The apparatus consists of all glass aspirator tubes set in individual glass-lined copper tubes containing mineral oil and immersed in a boiling water bath. The aspirator tubes do not touch metal at any point, thus minimizing the possibility of any rapid local transfer of heat at any point on the tubes, and precluding the occurrence of metal contamination when the tubes are cleaned. The water in the bath is maintained at a constant level by a minimum flow, so that local cooling at the point of intake will be negligible. The top of the bath is so constructed that the escaping steam does not come in contact either with the rubber tubing leading to the aspirators or with the long side-arms of the aspirators.

Compressed air is supplied by an aspirator pump which draws air from outside the laboratory, and is kept at a constant pressure by an escape system. The air then passes through three wash bottles containing a 2% solution of potassium permanganate in 1% sulphuric acid, a 20% solution of potassium hydroxide and concentrated sulphuric acid, respectively. The air is thus freed from water vapor and small amounts of gases such as carbon dioxide which would affect the indicator solution. The solutions are maintained at fixed heights in the wash bottles and are renewed at definite intervals. The amount of air passing into each of the fat tubes is regulated by pieces of thermometer tubing, as described below, and a check is kept on the total pressure, which should be the same for each experiment, by a water manometer inserted in the aeration train.

The indicator solution is prepared by dissolving 0.1 gm. of Brom Cresol Green (pH range 4.0-5.6) in 14.3 cc. of 0.1N sodium hydroxide and diluted to 250 cc. with water. This solution is diluted ten times and adjustment to the desired blue shade (pH 5.7) with the aid of color standards or a pH meter. Ten cc. aliquots of the indicator solution are placed in matched test tubes and the color which denotes the completion of the induction period is the first definite green shade (pH 5.2). Peroxide tests show that a uniform degree of rancidity in lard or shortening exists at this point. The indicator end-point may be checked with standards by photoelectric colorimetry or visually; an Evelyn colorimeter with 440 filter has been employed in this laboratory.

Duplicate samples of 8 cc. of molten fat are used in the test and if a solvent is used to introduce the antioxidant the same volume of solvent is also added to the control tube. Solvents containing water must be avoided, not only because of the effect of water on fat stability (7), but also because the water vapor tends to condense in the upper part of the aspirator and retard the passage of volatile products of decomposition. The effect of each solvent upon the indicator solution and upon the induction period must be determined; peroxide-free ethyl ether is the most satisfactory. At the beginning of each test the air pressure is increased gradually to prevent spattering of the fat into the upper portions of the aspirator during the rapid volatilization of the solvent.

To standardize the apparatus, equal amounts of fat are measured into the aspirator tubes and the lengths of the thermometer tubing leading to each aspirator adjusted in a series of experiments until the indicator solutions for all the samples change simultaneously. This is checked with fats of both long and short in-

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duction periods and the stability time is determined to the nearest five minutes. The regulation of the rate at which air passes through the samples is a somewhat troublesome procedure, and there is still some doubt as to whether this type of control is adequate. By this method of calibration the same volume of air may not pass through each sample in a given time, but the small variations compensate for any slight differences in the dimensions of the aspirators.

Absolute cleanliness of the apparatus is essential if uniform results are to be obtained. As much of the material as possible is removed from the aspirators by draining and steaming and then they are washed in chloroform or petrol ether, treated with hot concentrated nitric acid for several hours and finally rinsed in distilled water and dried in a hot-air oven at 120°C.

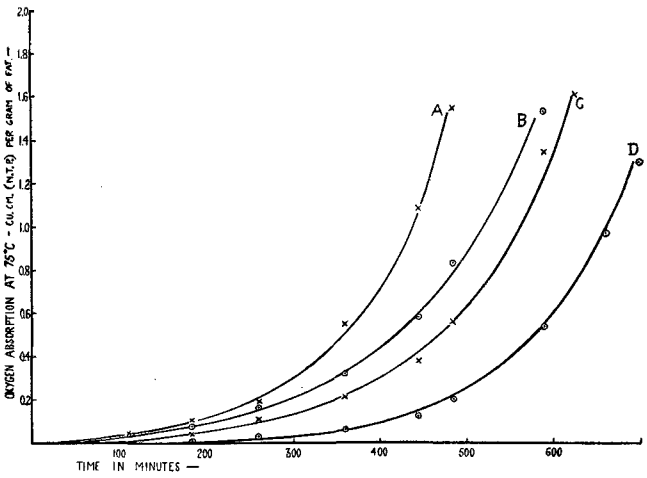

FIG. I. Showing the relative rates of oxygen absorption in Warburg respirometer by samples of vegetable shortening which had been stored for 4½ months at 33°F.

A—Control sample untreated.

B, C, D—Treated samples—Same vegetable fat treated prior to deodorization with (B) 0.05% wheat germ oil (C) 0.05% wheat germ oil—antioxidant formula B (D) 0.05% wheat germ oil—antioxidant formula C.

II. *Oxygen Absorption.* Oxygen uptake measurements were made with the Barcroft-Warburg apparatus, employing a technique similar to that of Johnston and Frey (5). The water-bath temperature was maintained at 75°C. by means of a mercury-toluene thermo-regulator. One gram of fat in a flask of approximately 35 cc. capacity was found to be convenient. Brodie's solution was used as the manometer fluid and the pressure measurements were evaluated in the usual manner, utilizing calibration data to convert pressure readings to volumes of oxygen absorbed. The flasks were cleaned in the same manner as the aspirators used in the Swift aeration test. Samples were run in duplicate, two flasks being employed as thermo-barometers.

III. *Peroxide Oxygen.* The ferric thiocyanate method recently developed in this laboratory for the study of rancidity in milk powders (2) was used to determine peroxides. In this method a reagent containing 0.1% ferrous ammonium sulphate and 0.4% ammonium thiacyanate in 96% acetone is added to a 1 cc. aliquot of an acetone solution of the fat and the red color of ferric thiocyanate, developed by heating for 10 minutes at 50°C. in a water bath, is measured in a Coleman Spectrophotometer.

This procedure gives more readily reproducible results than the usual peroxide titration methods and also measures more completely the extent of oxidation, particularly with highly unsaturated fats, and thus enables the course of rancidity to be followed very closely in storage tests (14).

Development of the Antioxidant

CHOICE OF A SUITABLE BASE OIL:

Wheat-germ oil possesses the desired properties for a base oil, however, the antioxidant activity depends on the method used to extract the oil from the germ. Wheat-germ oil extracted by means of ethylene dichloride has a particularly high antioxidant content (10). It is a potent source of the tocopherols which are natural antioxidants of the phenolic type; it has a high content of lecithin which also acts as an antioxidant; and studies conducted in this laboratory indicate that it also contains additional substances with stabilizing properties. Earlier work here has shown it to be much superior to cottonseed oil in the stabilization of bacon fat, and to have a much higher tocopherol content than corn, cottonseed and appleseed oils (12). The oil has a pleasing mild nutty flavor and its use does not affect the natural flavor, color and texture of fats and foodstuffs such as may be the case when finely divided vegetable material such as oat, barley, or soyabean flour is incorporated into foods as an antioxidant.

Table I shows the stabilization imparted by wheat germ oil to representative samples of lard, blended

TABLE I

The Stabilization of Fats and Oils by Straight Wheat-Germ Oil as Determined by a Modified Swift Test

Sample	Percent wheat-germ oil added	Stability Time (minutes)		
		Control	Treated	Difference
Bleached lard				
No. 1	0.01	120	130	10
No. 2	0.05	100	160	60
No. 3	0.10	220	320	100
No. 4	0.20	140	330	190
Prime steam lard				
No. 1	0.01	210	210	0
No. 2	0.10	140	270	130
No. 3	0.10	130	265	135
No. 4	0.10	85	130	45
No. 5	0.10	105	155	50
Blended shortening				
No. 1	0.02	180	235	55
No. 2	0.05	145	220	75
No. 3	0.05	360	480	120
Oleostearine	0.10	610	965	355
Peanut Oil	0.10	820	880	60
Olive Oil	0.05	390	420	30

shortening and other materials. The added stabilization obviously is not directly related to the induction period and will vary with the composition of the fat, the method of processing, the degree of removal or destruction of the natural antioxidants, and the possibility of synergistic action of added stabilizers with the residual natural antioxidants. The antioxidant should exert its maximum effect if it is added before the fat shows any trace of spoilage, or if it is added to the fat or oil before it is deodorized.

FORTIFICATION OF WHEAT-GERM OIL:

The problem of finding an acidic substance which would enhance the activity of wheat-germ oil was next considered. Of the naturally occurring acids which

had some degree of fat solubility, ascorbic, tartaric and citric seemed to be the most promising. The synergism of ascorbic acid with α-tocopherol was demonstrated by Golombic and Mattill (3), and tartaric acid and citric acid have been shown to be definitely superior to maleic acid in the stabilization of esters of hydrogenated cottonseed-oil, and to enhance the activity of wheat-germ oil concentrates (9). Citric acid has the advantage over tartaric acid of being appreciably more soluble in fat solvents.

Table II shows the comparative antioxidant potencies of wheat-germ oil and wheat-germ oil fortified with ascorbic acid and also indicates that the action of ascorbic acid may be duplicated by citric acid. Practical considerations led to the selection of citric acid as the fortifying agent.

TABLE II

Showing the Comparative Antioxidant Potency of Wheat-Germ Oil and Wheat-Germ Oil Fortified With Organic Acids as Determined by the Modified Swift Test

Sample	Stability time	Increase over control
	minutes	*minutes*
Bleached lard control	150	.....
Control + 0.2% wheat-germ oil	330	180
Control + 0.1% wheat-germ oil containing 0.5% ascorbic acid	325	175
Bleached lard control	100	.....
Control + 0.1% wheat-germ oil	160	60
Control + 0.1% wheat-germ oil containing 0.5% ascorbic acid	200	100
Control + 0.1% wheat-germ oil containing 0.5% citric acid	200	100
Bleached lard control	180	.....
Control + 0.05% wheat-germ oil	235	55
Control + 0.05% wheat-germ oil containing 1.0% citric acid	290	110
Prime steam lard control	90	.....
Control + 0.1% wheat-germ oil	130	40
Control + 0.1% wheat-germ oil containing 2% citric acid	360	270

Preliminary work indicated that the maximum amount of citric acid which could be held in solution in wheat-germ oil under ordinary conditions of incorporation was of the order of 2-5%. However, a method was devised whereby the citric acid content could be increased to 10%. In view of the low optimum concentration of citric acid (Table III), a preparation containing 5% of citric acid (monohydrate) was finally established as being the most satisfactory. No separation of citric acid from this mixture occurred even upon chilling to 0°C.

TABLE III

The Optimum Concentration of Citric Acid Alone, as Determined by the Modified Swift Test

Sample	Percent added citric acid	Stability time	Increase over control
		minutes	*minutes*
Bleached lard	0.0	85	.....
	0.02	215	130
	0.04	200	115
	0.10*	205	115
Blended shortening	0.0	520	.....
	0.005	615	95
	0.04	615	95
Blended shortening	0.0	170	.....
	0.001	310	140
	0.002	350	180
	0.003	350	180
	0.004	380	210
	0.005	360	190
	0.006	340	170
	0.007	340	170
	0.008	325	155
	0.016	325	155

* Crystallization of citric acid from the lard occurred at this concentration.

The preparation, known as Formula "C," is most effective in stabilizing fats of poor keeping quality. Laboratory tests indicate an optimum concentration of 0.01% in typical blended shortening of low stability, and at this concentration it is superior to equivalent concentrations of gum guaiac and hydroquinone (Tables IV and V). Collaborative work by commercial firms has shown that the material is most effective when added prior to deodorization and that the optimum concentration is then of the order of 0.05 to 0.10%.

TABLE IV

Optimum Concentration of Formula "C" in a Typical Blended Shortening of Low Stability as Determined by the Modified Swift Test

Percent of added antioxidant	Stability time	Increase over control
	minutes	*minutes*
.....	220	.....
0.005	460	240
0.01	555	335
0.02	550	330
0.04	560	340
0.08	555	335

TABLE V

The Antioxidant Activity of Formula "C" Compared to That of Gum Guaiac and Hydroquinone as Determined by the Modified Swift Test

Antioxidant	Stability time	Increase over control
	minutes	*minutes*
Nil control	150	....
0.01% gum guaiac	165	15
0.01% hydroquinone	225	75
0.01% formula "C"	385	235

Formula "C" is now being prepared on a commercial scale* by a method substantially similar to the one which was developed in this laboratory, and the commercial samples have essentially the same potency as the material prepared on a laboratory scale.

PRACTICAL APPLICATION:

The practical value of Formula "C" has been established by collaborative experiments with commercial firms. A large packing house in the United States was supplied with the following antioxidant materials:

Oil A Straight wheat-germ oil.

Oil B Wheat-germ oil containing 2 percent citric acid.

Oil C Formula "C."

Oils B and C were prepared in this laboratory and the company was not informed of the composition of the oils, nor did they know which oil had been fortified with additional antioxidant. A preliminary report from this firm on samples "A" and "B" reads as follows:

"The Samples . . . were treated on a small scale in the laboratory in the stabilization of mixed type shortenings. 0.05% of sample 'A' increased a shortening with an original active oxygen of 21 hours to 35 hours, while 0.1% increased it to 37 hours. The shortening was deodorized after the wheat-germ oil was added. No increase in color was obtained. 0.05% of sample 'B', added to the same original shortening, produced a stability of 49 hours, while 0.1% produced a stability of 50 hours. Again, no increase in color was noted.

* "Viobin Antioxidant," manufactured by the Viobin Corporation, Monticello, Illinois.

"Tests were made on lard on a small scale in the laboratory. 0.05% and 0.10% of samples A and B increased a 5 hour lard to 6 hours. One percent of each sample increased it to 23 hours."

A later report presented essentially this information:

"The antioxidant materials were tested in our pilot plant in a shortening made from a blend of meat food fats and unhydrogenated vegetable oils. About 2,000 pounds of shortening could be handled in the pilot plant, so the test was on a semi-commercial scale. Laboratory work showed that the best results were obtained if the antioxidants were added prior to deodorization, so this plan was followed. After deodorization, the products were passed over a chilled roll and packaged in the same manner as they would be handled commercially. Our 'active oxygen stability' results on the shortenings thus prepared are as follows:

	A. O. M.
Control	7 hours
Control + 0.05% sample 'A'	12 hours
Control + 0.05% sample 'B'	16 hours
Control + 0.05% sample 'C'	25 hours."

Comparable results were obtained in this laboratory when these shortenings were tested by the modified Swift Test. The relative stability of these samples was also checked by oxygen absorption and peroxide measurements (Table VI) after long periods of storage.

TABLE VI

Peroxide Oxygen Content (Ferric Thiocyanate Method) of Stabilized Blended Shortening Prepared by a United States Packing Firm, After Storing 7½ Months at 33° F. and Subsequently 1½ Months at Room Temperature

Sample	Peroxide value
	milli-equivalents of peroxide per kg.
Control	33.8
Control + 0.05% sample "A"	32.4
Control + 0.05% sample "B"	20.5
Control + 0.05% sample "C"	7.4

Through the cooperation of a Canadian firm a similar experiment was carried out with lard, the antioxidant being added in this case before the lard was rendered. The samples were held at 20-25°C. and then tested with the results shown in Table VII.

Baking and deep-fat frying tests by the School of Household Science, Macdonald College, showed no difference in flavor and other properties between control samples of lard and blended shortening and samples containing Formula "C."

TABLE VII

Stabilization of Lard on a Commercial Scale

Sample	Stability time Modified Swift test	Peroxide value Ferric thiocyanate method
	minutes	*m.e. per kg. After aging 30 days*
Raw lard control	65	61.8
Raw lard + 0.055% Formula "C"	190	14.6
Finished lard control	60	87.1
Finished lard + 0.055% Formula "C"	105	23.7

Summary

The process of developing a practical antioxidant consisting of natural occurring food substances has been described. The efficiency of the final product, which is based upon wheat-germ oil extracted from wheat-germ by means of ethylene dichloride and which contains added citric acid, has been verified by means of a modified Swift test, oxygen absorption and peroxide estimations. The practicability of the antioxidant has been demonstrated on a plant scale.

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