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8. OXIDATIVE RANCIDITY AND ANTIOXIDANTS

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Oxidative rancidity is a well known type of spoilage which occurs in fats and oils and fat components of foods. It results from the action of atmospheric oxygen with the fat or oil molecules and is characterized by a typical, strong and disagreeable odor and flavor. The oxidation or rancid products not only make the food unpalatable but also destroy certain vitamins. The deleterious action of atmospheric oxygen is not restricted solely to fats. Many organic compounds and substances—particularly those with unsaturated bonds or branched chains, such as are found in petroleum products and rubber—undergo similar autoxidation. In many instances the same agents which accelerate or retard the oxidation of fats will have a similar effect on these various organic substances. Hence the mechanism and control of atmospheric oxidation are of widespread interest and importance.

Mechanism of Autoxidation

Some means of envisaging the mechanism of autoxidation is essential for understanding how various factors and agents may accelerate or retard the reaction. The study of the mechanism, products formed, and factors that influence the oxidation has been the subject of a great many investigations for more than fifty years. Considerable progress has been made and an acceptable mechanism has been proposed.

Many of the earliest observations on reactions with molecular oxygen or other gases, and ideas concerning the mechanism resulted from investigations on substances other than fats, such as hydrocarbons, alcohols, and aldehydes. The suggestion that a peroxide is the primary oxidation product

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formed in the direct reaction of molecular oxygen with a substance originated with Bach[12] and Engler and Wild[83] in 1897 although the first peroxide was isolated in 1900[14]. The earliest attempt to explain the mechanism is attributed to Arrhenius[10], who in 1889 proposed the concept of "activated" molecules. He suggested that there is an equilibrium between normal molecules of a gas and active molecules, the latter being fewer in number but possessed of much greater energy than the average and being the only ones capable of reaction. In 1913, Bodenstein and Dux[34, 35] proposed the theory of chain reactions which, with modifications such as the free radical concept of chain initiation and propogation, is generally accepted at the present time. The early work in this field has been reviewed by Bailey[17].

The concept of free radicals probably had its origin in the well-known work of Gomberg who prepared triphenyl methyl[120, 121] and explained the preparation and properties of hexaphenyl ethane on the basis of a free radical reaction[122]. The concept of free radicals has since been frequently applied to explain reactions of the Wurtz-Fittig type, Grignard reactions, photochemical reactions, and many others.

The application of the concept of free radicals and chain reaction to the autoxidation of olefines and polyolefines, including fats and oils, is credited chiefly to a group of British workers[93, 94, 95, 96, 97, 98, 99, 42]. Since a number of reviews of this work have been published recently[215, 290, 253, 187, 39, 40, 309] only a summary of the theory and pertinent findings in relation to the problem of oxidative rancidity will be attempted here.

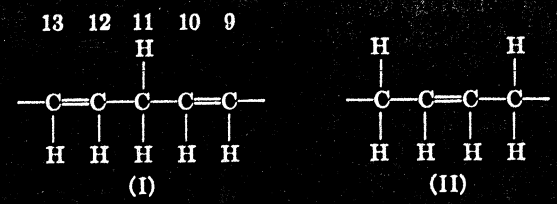
TABLE 8-1. OXYGEN ABSORPTION OF METHYL ESTERS OF FAT ACIDS AT 100°C[302]

Substrate		Oxygen absorption	
% Methyl esters	Iodine number	Minutes required to absorb 1 g./kg*	G./kg. at 60 min.
100 linolenate	260.4	7	19.1
100 linoleate	172.4	11	10.2
90 linoleate, 10 stearate	155.2	13	10.0
80 linoleate, 20 oleate	155.0	15	8.4
50 linoleate, 50 oleate	129.0	17	6.6
50 linoleate, 50 stearate	86.2	18	5.0
25 linoleate, 75 stearate	43.1	35	2.6
20 linoleate, 80 oleate	103.0	36	2.9
100 oleate	85.6	115	.2
85 oleate, 15 stearate	72.8	166	.2
57 oleate, 43 stearate	48.8	337	.1
100 stearate	0	1250	.05

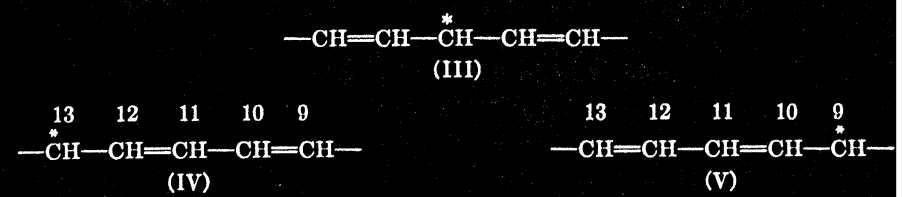
* Amount of absorbed oxygen corresponding approximately to the organoleptic rancid point of most fats.

The polyunsaturated components of fats oxidize many times faster than do the monoetheonoid and saturated acids[147, 170, 318, 302, 148]; in fact, so much faster it is unlikely that any but the polyunsaturated components undergo autoxidation in time required for normal fats to become rancid. The data in Table 8-1 support this conclusion. Some doubt has even been expressed[144] that highly purified methyl oleate undergoes autoxidation at temperatures below 50°C. Linoleic acid is one of the principal acids of our common edible fats—lard, and soybean, cottenseed, peanut, and corn oils. Acids of greater unsaturation, linolenic and arachidonic acids, are often present in minor proportions. Hence, the mechanism of autoxidation of linoleic acid is of prime importance in oxidative rancidity.

These polyunsaturated acids have in common a methylene interrupted system of double bonds as shown (I) for the pentadiene system of linoleic acid. The two double bonds flanking Carbon 11 greatly increase the lability of the hydrogen atoms on this carbon atom as compared to the influence of one double bond on the adjacent methylenes in oleic acid (II)



Sufficient energy is acquired as heat or light energy to permit the removal of a labile hydrogen from the active methylene of (I) by molecular oxygen which has unpaired electrons. Momentarily there is produced an intermediate unstable radical (III) which immediately undergoes electronic stabilization to form the conjugated resonance hybrids (IV) and (V)



An oxygen molecule adds to Carbon 13 (IV) or Carbon 9 (V) forming peroxide radicals which extract hydrogen from the active methylene of an adjacent molecule of (I), thereby producing a new radical (III → IV, V) and hydroperoxide. For convenience the radical chain reaction mechanism may be represented by the following oversimplified steps, where RH = (I) and R* = (III) → (IV) and (V)

$$(1)\quad RH + {}^{*}O{-}O^{*} \xrightarrow[\triangle \text{ or light}]{} R^{*} + {}^{*}OOH \qquad \text{(free radical initiating)}$$

$$\left.\begin{array}{ll} (2) & R^{*} + {}^{*}O{-}O^{*} \longrightarrow RO_2^{*} \\ (3) & RO_2^{*} + RH \longrightarrow RO_2H + R^{*} \end{array}\right\} \text{chain propagating}$$

$$\left.\begin{array}{ll} (4) & R^{*} + {}^{*}OOH \longrightarrow RO_2H \\ (5) & R^{*} + R^{*} \longrightarrow RR \\ (6) & R^{*} + RO_2^{*} \longrightarrow RO_2R \end{array}\right\} \text{chain terminating}$$

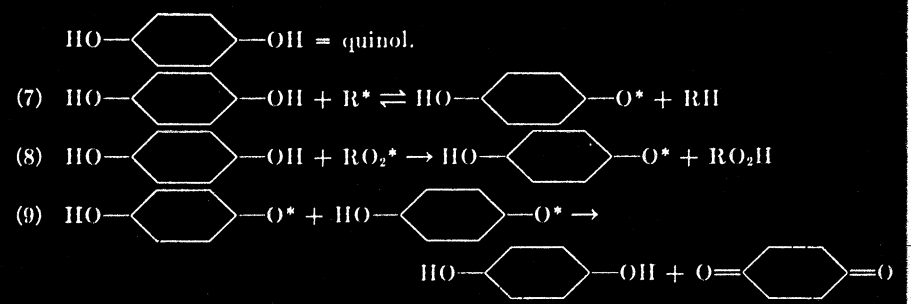
The primary oxidation product, hydroperoxide, derived from (IV) or (V) is conjugated as shown conclusively by several investigators[96, 188, 51, 287] and in the early stages accounts for nearly all oxygen absorbed; about 90 per cent of the isolated peroxides are conjugated hydroperoxides. As the oxidation proceeds the conjugated hydroperoxides may undergo further oxidation and form diperoxides by 1,4 addition[75, 188], the latter decomposing into products such as aldehydes, acids, and ketones. The hydroperoxides may react (*chemical* oxidation) with double bonds of nearby molecules[263, 311], thus accounting at least in part for the rapid loss of unsaturation and corresponding decrease in peroxides during advanced stages of autoxidation. It is evident that a multiplicity of reactions are occurring simultaneously with the primary autoxidative mechanism and producing a mixture of many oxidation products which increase in complexity as the oxidation proceeds. Some decomposition of peroxides must occur even during the "prerancid" induction period because the so-called "reverted" odors and flavors precede the detection of typical rancidity. "Reversion," however, appears to be related to the effect of metallic impurities on the autoxidation of linolenic acid principally; the products resulting could be different from those of typical rancidity, although some of them seem to be the same[311, 312, 45, 281, 298, 156].

Role of Antioxidants and Catalysts

It is clear that the absorption of heat or light energy would accelerate any of the steps represented in the autoxidation although not necessarily to the same extent. Very little is known about the kinetics of the oxidation steps, although recent work[39] has shown a parallellism between rate expressions for reactions between oxygen and ethyl linoleate accelerated by (a) benzoyl peroxide, (b) illumination with ultraviolet radiation, and (c) autoxidation with oxygen in the dark. It was concluded that in either case, the chain propagating and terminating mechanisms were similar but there were divergencies in modes of chain initiation.

Metal salts or oxides, particularly those of the heavy metals, such as copper and iron, are powerful catalysts and affect the nature of the oxidation products[63, 107]. Absorbed light energy is known to be a powerful initiator and promotor of free radical mechanisms and to give high quantum yields of product[13, 4, 35, 34, 58].

No definition of antioxidant based on mode of action in retarding the primary autoxidation by means of radical or chain termination has been universally accepted, probably because too little is known about the mechanism. Suggestions have been made as to the possible retarding action of quinols, including tocopherols[62, 101, 211, 116, 210]. The mechanism may be represented somewhat as follows: where RH = linoleic pentadiene system with H as the labile hydrogen, R^{*} = free radical, RO_2^{*} = oxygenated free radical, RO_2H = primary product (hydroperoxide), and



Steps (7) and (8) indicate that the antioxidant furnishes hydrogen atoms to terminate the initiating and propagating mechanism of autoxidation. Owing to the energy required to remove hydrogen from RH to form R^{*}, this step would be sluggish, and if the antioxidant were present at the start before many radicals and chain carriers were produced, it is understandable how only trace amounts could retard greatly the progress of autoxidation even though eventually the antioxidant is destroyed. Evidence that semiquinone radicals can be formed and are stable enough to exist for appreciable time has been published[62, 101, 211]. Experimental evidence[114, 102] has shown that antioxidants are destroyed during the induction period following which there is a rapid increase in the rate of peroxide formation.

It is inadequate to say that an antioxidant is any added substance which retards or decreases the rate of autoxidation, because the rate of oxidation of a substrate containing prooxidant metal salts may be decreased considerably by the addition of certain metal complexing (deactivating) agents. Likewise, since the oxidation is autocatalyzed by hydroperoxides formed, the rate may be decreased by reducing the peroxides with a reagent such as a sulfite.

A number of polyhydroxy- or acidic compounds, among which are ascorbic, citric, tartaric, galacturonic, and phosphoric acids bring about a marked

enhancement of the stability of fats containing phenolic antioxidants. These compounds have been referred to as synergists, and the enhancement as a synergistic effect[241, 118, 49, 200, 50, 266]. In the light of more recent work they appear to have metal deactivation properties in fats and the enhancement can for the most part be explained by the latter function. More will be said about synergists and metal deactivators later in the chapter. The term synergists will be used in these discussions at times without distinction as to mode of action.

Ascorbic acid or its fatty acid monoester[269, 310] could be considered as a borderline type in that it appears to function as a metal deactivator[217] and yet has some of the characteristics of a synergist and antioxidant. The similarity between the endiol group $-\overset{OH}{\overset{|}{C}}=\overset{OH}{\overset{|}{C}}-$ in ascorbic acid and in catechol, and to a lesser extent in hydroquinone suggests possible similarities in their oxidation-reduction mechanism[335]. Possibly the oxidation of ascorbic acid, like hydroquinone, is a univalent free-radical two-stage mechanism,

$$-\overset{OH}{\overset{|}{C}}=\overset{OH}{\overset{|}{C}}- \rightleftharpoons -\overset{O^*}{\overset{|}{C}}=\overset{OH}{\overset{|}{C}}- + H^* \text{ and } -\overset{OH}{\overset{|}{C}}=\overset{O^*}{\overset{|}{C}}- \rightarrow -\overset{O}{\overset{\|}{C}}-\overset{O}{\overset{\|}{C}}- + H^*$$

when used with phenolic antioxidants. If so, it functions in a manner similar to that of phenolic inhibitors in fats, or in other words, it inhibits the oxidation of quinols by furnishing hydrogen atoms to the semiquinone radicals thus regenerating the quinol (the primary fat antioxidant)[50, 119, 149]. The possible relation of oxidation potentials of antioxidants and synergists to their effectiveness has been suggested[116]; the most effective antioxidants falling in the potential region between 848 and 484 millivolts. In the above system the oxidation potentials would be in the following order: fat peroxides > quinol > ascorbic acid.

Phosphoric acid also functions as a metal deactivator[217] and apparently regenerates tocopherol and quinol (or phosphorylated product which acts as an inhibitor) from quinones or semiquinones[49].

Stability Tests

Most fats and oils when fresh have a slight but characteristic natural flavor. Upon aging exposed to air they begin to lose their fresh flavor and acquire "off" flavors described variously by such terms as stale, musty, beany, fishy, painty, tallowy, etc., until the product becomes rancid. The rancid odor and flavor resemble that of heptylic and nonylic aldehydes. The off-flavors developed prior to the rancid stage, i.e., during the so-called induction period, are often referred to as reverted flavors (or odors) and undoubtedly are caused by atmospheric oxidation, because during this period hydroperoxides (or peroxides) are slowly being produced and natural antioxidants such as tocopherols are being destroyed as are also vitamin A and carotene. The most highly unsaturated fatty acid components would be the first to start autoxidizing. Animal fats, for example, contain a few tenths of a per cent of acids with four and five double bonds[142]. The oxidation of these minor components could easily account for reverted flavors during the pre-rancid stage; the peroxides formed may oxidize certain trace components responsible for the original fresh flavor. By the time the fat or oil is rancid, the inhibitors are destroyed and volatile cleavage products such as aldehydes are being produced. Only a few parts per million are required for detection by sense of smell or taste[22]. It has been reported that definite rancidity can be detected when 1/10 of a per cent of fat has undergone oxidative change[183]. Calculations based on peroxide values at the usual rancid point, however, indicate that more nearly 1 per cent of the glycerides would be affected.

Like most subjective tests, organoleptic detection of rancidity suffers a number of disadvantages. Individuals differ in their sensory acuity; even the same person may vary in this respect from time to time. Such tests are not amenable to quantitative expressions that could well be used in following the progress of the oxidation. They are helpful, however, as confirmatory tests in conjunction with chemical tests. Some laboratories have established elaborate "taste-panels" for detection of degree of reversion. Methods and suggestions for conducting such panels have been published[70, 159, 213, 220, 196]. Panel grading, however, requires a great deal of time and is not well adapted to routine testing.

A number of chemical tests for detection of the rancid point based on colorimetric measurements of aldehydes or carbonyl compounds have been used rather extensively[168, 157, 146, 259, 177, 100, 254, 282]. The intensity of color or amount of carbonyl taken as the rancid point should be calibrated with the organoleptic test. Since the formation of carbonyls parallels peroxide formation and oxygen uptake during the early stages of autoxidation, it has become a more general practice to use either of the latter measurements to indicate the end of the induction period. This appears not unreasonable because oxygen absorption and peroxides are precursors of the rancid products, and are relatively easy to measure quantitatively. None of these tests for rancidity are reliable for all food products particularly those subjected to unusual conditions. For example, fats being used in deep fat frying do not show a high level of peroxide because the peroxides decompose rapidly at such temperatures. Similarly the decomposition products, i.e., aldehydes, are rapidly removed through volatilization. The state of oxidation in some instances can only be determined by comprehensive analyses of changes

in fatty acid composition, unsaturation, viscosity, and by determination of various oxygenated products.

Methods for detecting rancidity are essential in determining the stability of fats or fats in foods. Stability is defined as the resistance to autoxidation under prescribed conditions of aging and is measured in units of time required for the product to acquire a state of oxidation which correlates with organoleptic detection of rancid odor and flavor. Under usual conditions of storage or merchandising, this time is often too long for practical testing, particularly when antioxidants are being evaluated. Hence it is necessary to employ accelerated conditions of aging. Lea[178] has reviewed the literature on rancidity in edible fats up to 1937 and has discussed the detection of rancidity and stability tests in greater detail.

Stability by Active Oxygen Method. The most widely used method for determining stability of fats and oils is known generally as the Active Oxygen Method (AOM), although it is also frequently called the Swift Stability Test. The essential features of the method were first described by Wheeler[336]. A modified and simpler form of the apparatus and technique described by King, Roschen and Irwin[158] has been adopted as standard by many laboratories in this country. Several minor modifications[267] have also proved helpful. A uniform flow of washed air is bubbled through the sample of fat contained in an aeration tube, which is immersed in an oil bath maintained at constant temperature, 98 to 99°C, by a boiling water jacket. The progress of autoxidation is followed by periodic organoleptic tests and peroxide determinations. A record of the time is kept from the beginning of the test and for each test period until the sample is definitely rancid. From curves showing a plot of time vs. peroxide accumulation, the time required for the samples to attain the peroxide level established as the "endpoint" can be estimated. Under these conditions rancidity in meat fats can usually be detected organoleptically at a peroxide level of 20 meq. of peroxidic oxygen per kilogram of fat, whereas for vegetable oils and shortenings it is customary to use a peroxide value of 100 meq./kg. as the endpoint. When antioxidants are added to lard, particularly in concentrations of 0.05 per cent or greater, the end of the induction period, as indicated by the inflexion of the time-peroxide curves and also by organoleptic tests, occurs at a considerably higher peroxide level than 20 meq./kg. Hence it has been suggested[214] that a uniform peroxide value of 100 be taken as the rancid point for lard as well as for vegetable fat. A diagram of an aeration tube, air-distributing system, and a photograph of the assembled apparatus are shown in Figures 8-1, 8-2, and 8-3; typical time-peroxide curves in Figures 8-4 and 8-5.

With minor modifications of the bath and a different type of aeration tube, the apparatus has been adapted to the determination of stability of

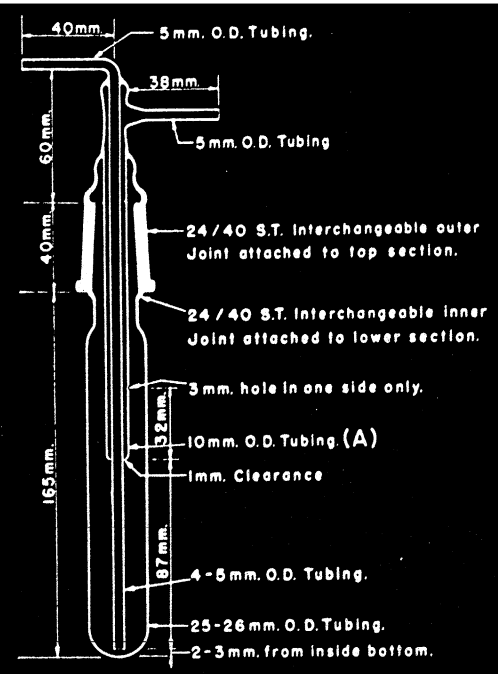


Figure 8-1. Diagram of aeration tube for use in determining stability of fats by the active oxygen method[267]. (A) Device to prevent foam from being carried out of tube.

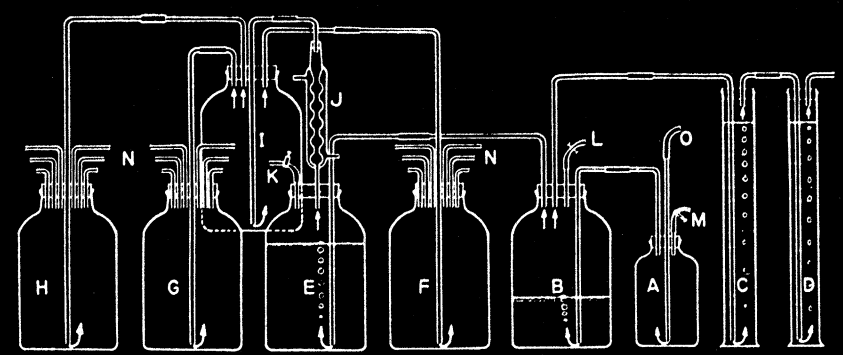


Figure 8-2. Diagram of air purification and distributing system for use in active oxygen method[267]. (A) device to control pressure of incoming air; (B) bottle containing water for washing air; (C), (D) water columns. Air in space above water in (B) is kept under constant pressure sufficient to by-pass air through (C) and (D) at a steady rate; (E) bottle containing acid dichromate solution. The air in (E) passes through condenser (J) into bottle (I), then into bottles (F), (G), and (H) which distribute the air to the tubes (N), which lead to the aeration tubes. (M) screw clamp to regulate flow of air; (O) connection to source of air pressure; (K), (L) pinchcocks to release pressure when shutting off apparatus.

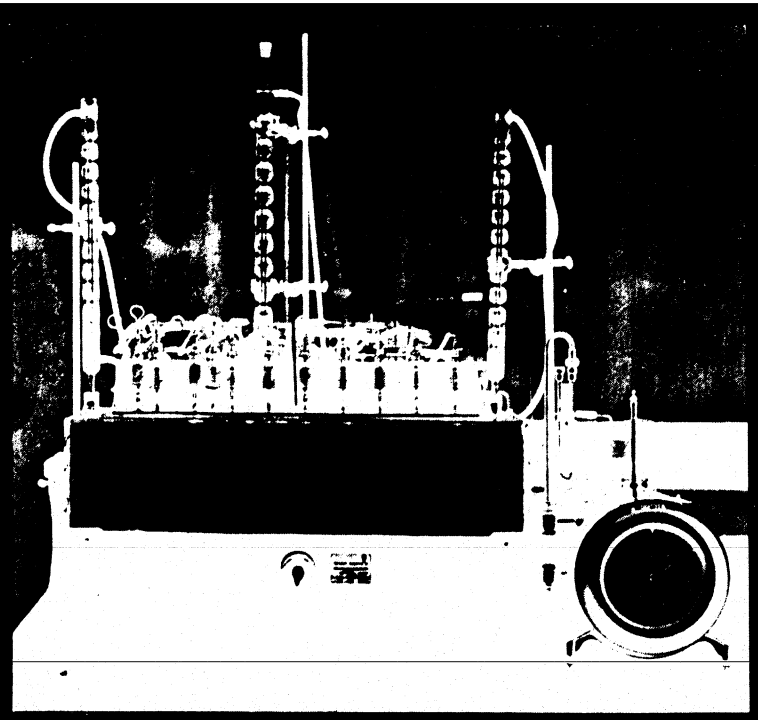


Figure 8-3. Assembled apparatus for determining stability by the active oxygen method.

dry dog meals[233]. The relative concentration of carbonyl compounds in the exit gases from the meal sample being tested were determined colorimetrically by a modification of the procedure of Lappin and Clark[173]. There was a linear relation between formation of carbonyl and peroxide. It seems probable that this modification could be used to determine the stability of many dry feeds and cereal products.

Stability by Oxygen Absorption Methods. Oxygen uptake measurements have been used in many of the earliest studies of autoxidation of oils[108, 61, 146], and in evaluation of antioxidants[221]. The method is still preferred by some workers. Relatively simple apparatus [125, 106, 314, 154, 328] appears to be adequate. For strict comparison of results between different laboratories, however, standard apparatus and procedure should be adopted. The time required for the sample to absorb a pre-established amount of of oxygen under the specific conditions of the test is taken as the stability. This value will vary considerably with different size samples, degree of agitation, temperature, and other factors. One disadvantage of oxygen absorption methods is that organoleptic tests cannot readily be made at intervals during the period of oxygen uptake. An example of the type of apparatus used by Mattill and co-workers is illustrated in Figure 8-6.

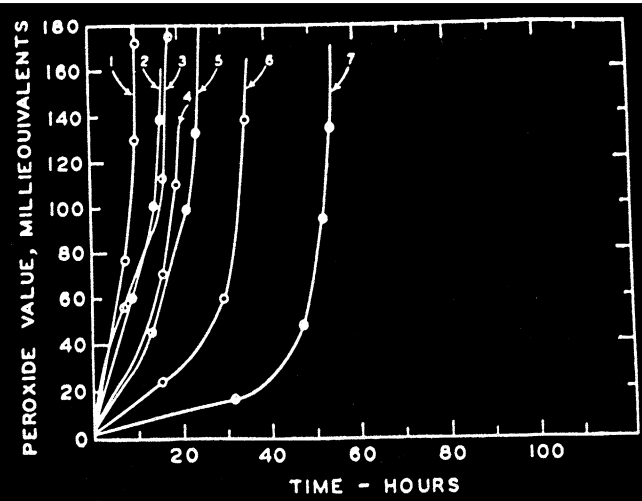


Figure 8-4. Typical time-peroxide curves obtained in determining stability by the active oxygen method[214]. Cottonseed oil containing 0.05% antioxidant. (1) Control; (2) ditertbutyl-*p*-cresol; (3) sesamol; (4) nordihydroguaiaretic acid; (5) norconidendrin; (6) hydroquinone; (7) propyl gallate.

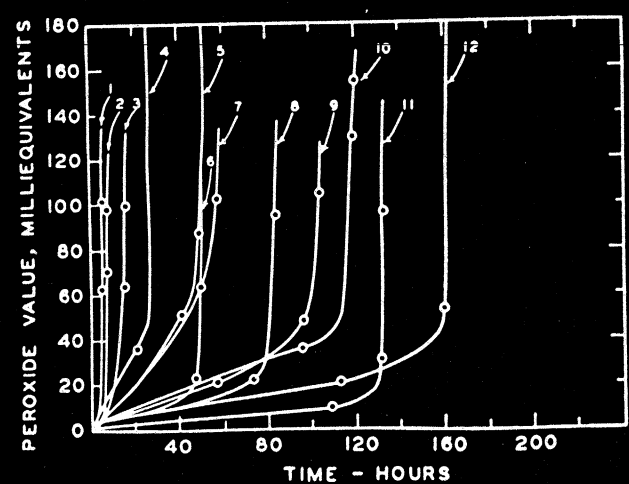


Figure 8-5. Typical time-peroxide curves obtained in determining stability by the active oxygen method[214]. Lard containing 0.05% antioxidant. (1) Control, (2) lecithin; (3) gum guaiac; (4) α-tocopherol; (5) α-tocopherol; (6) ditertbutyl-*p*-cresol; (7) butylated hydroxyanisole; (8) sesamol; (9) norconidendrin, (10) nordihydroguaiaretic acid; (11) hydroquinone; (12) propyl gallate.

Typical oxygen absorption curves on different commercial fats and oils are given in Figure 8-7.

Stability by Oven Incubation Method. Probably the simplest of the commonly used methods for determining stability of fat and some fatty

foods consists of heating the sample in a thermo-regulated oven and testing it at intervals for rancid odor and flavor, or for peroxide content. No standards for this method have been adopted, hence no strict comparisons can be made between results reported by different laboratories. The rather

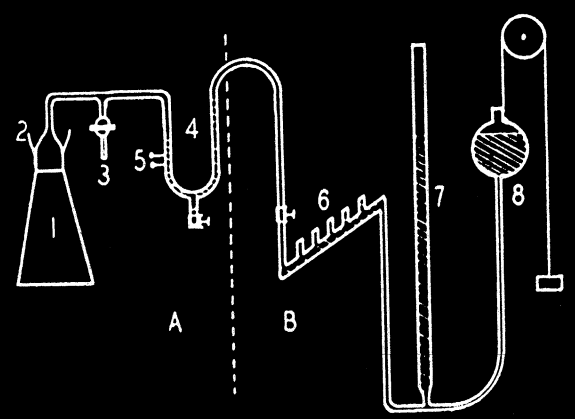


Figure 8-6. Oxygen absorption apparatus[106].

(A) System for determining length of induction period: (1) 250-cc flask with ground-in glass connection; the flask is immersed in a stirred, constant-temperature bath (not shown); (2) tip for mercury seal; (3) side arm for introducing oxygen; (4) Y-manometer; (5) electrical contacts for indicating pressure decrease when mercury is used in manometer.

(B) System for determining pressure changes in (A) at desired intervals: (6) common connections for battery of flasks; (7) 50-cc buret; (8) counterpoised leveling bottle; the fluid (water or Brodie's solution) is shaded in the glass parts and unshaded in the rubber tubing of the manometric system.

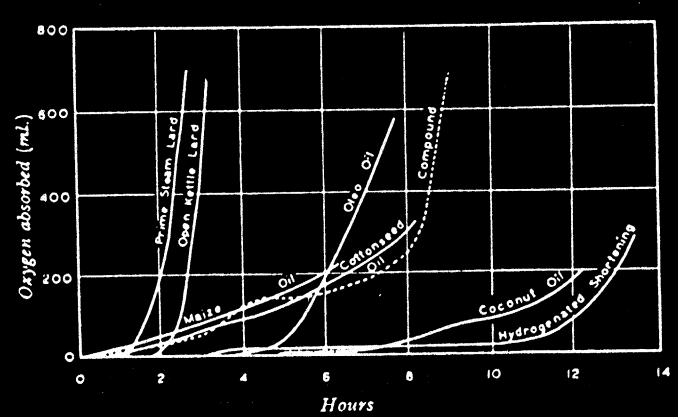


Figure 8-7. Typical time-oxygen absorption curves of commercial oils, lard, and shortening. 200 g samples stirred in oxygen at 95° C[327].

Table 8-2. Stability of Cottonseed and Soybean Oils Containing Added Antioxidants*

Antioxidants added	Conc.	Stability			
		AOM		Oven test at 140°F	
		Stabilized sample	Control	Stabilized sample	Control
	%	hr	hr	days	days
Cottonseed oil† + gum guaiac	0.10	19	15	14	12
+ propyl gallate	0.05	45	12	35	10
+ tocopherols‡	0.05	19	19	12	12
+ NDGA	0.02	14	12	12	10
Soybean oil† + gum guaiac	0.10	17	14	12	9
+ propyl gallate	0.05	54	18	45	14
+ tocopherols	0.05	20	19	12	11
+ NDGA	0.02	26	13	18	12

* Taken from published data[113].
† Refined and bleached.
‡ Commercial concentrate of natural tocopherols.

general conditions known as the Schaal test are most frequently employed[252, 130]. A 50-g. portion of fat contained in a 6 to 8 oz. wide-mouth glass jar covered with a watch-glass is heated in a constant temperature air oven at 140 or 145°F. The time required for the sample to become organoleptically rancid is recorded as the stability.

Oven tests at this temperature are also frequently used to determine the stability of fats in baked crackers and piecrust. Details of the preparation and baking of experimental quantities of piecrusts and soda crackers and their stability evaluations have been described[328, 38, 216, 143, 190]. Typical examples of data obtained by this method are shown in Tables 8-2 and 8-3.

Stability by Storage and Shelf-life Tests. Some fat-containing food preparations, particularly emulsified products, such as salad dressing, mayonnaise, cheese spreads, etc., are not amenable to the accelerated tests described. For these products room or cabinet storage tests, preferably at controlled temperatures, are desirable, the selection of temperature depending on the nature of the product. The detection of rancidity in such products often presents difficulties owing to the influence of flavoring agents and food components other than fats. Taste panel scoring and tests for aldehydes and peroxides are frequently used. It is usually necessary to extract a portion of the fat or oil for determination of aldehyde or peroxide. Since less than a gram of fat is required[267], in many instances the extraction can be made simply by grinding a suitable size sample of the food

TABLE 8-3. STABILITY OF LARD CONTAINING ADDED ANTIOXIDANTS AND STABILITY OF CRACKERS MADE WITH STABILIZED LARD*

Antioxidants added	Conc.	Stability		
		Lard		Crackers, oven test at 140°F
		AOM	Oven test at 140°F	
	%	*hr*	*days*	*days*
Lard (control, prime steam)	None	6	7	5
Gum guaiac	0.01	10	9	12
Gum guaiac	.05	20	22	30
Gum guaiac	.10	24	23	35
Lard (control, prime steam)	None	6	7	8
Propyl gallate	0.01	33	30	11
Propyl gallate	.03	50	60	12
Propyl gallate	.05	135	124	13
Propyl gallate	.10	145	135	14
Lard (control, prime steam)	None	4	4	9
Tocopherols†	0.02	16	15	11
Tocopherols	.10	23	18	12
Lard (control, prime steam)	None	5	6	11
NDGA	0.01	18	25	25
NDGA	.02	35	32	32
NDGA	.05	45	35	35

* Taken from published data[113].
† Commercial concentrate of natural tocopherols.

product with anhydrous sodium sulfate in a mortar and triturating with several portions of anhydrous peroxide-free ethyl ether, and filtering. The ether is removed by warming under the reduced pressue of a water pump, leaving the oil as a residue.

Storage tests at room temperature have also been employed for meat fats and shortenings, usually as a final confirmatory test after evaluations by other accelerated stability and performance tests have been conducted.

General Discussion of Stability Tests. Owing to the many variables in products and uncontrolled factors in methods for determining stability, it is impossible to make strict comparisons of much of the published data or to give satisfactory quantitative expression for the relative values of antioxidants. The principal uses of the accelerated tests are for rapid guides in quality control of the product, and for preliminary "screening" of antioxidants and experimental processes.

In much of the published work on antioxidants, it has been common practice to describe the test used, and give stability values for the control fat and for the fat containing a stated concentration of antioxidant; some workers prefer to express the results relating to the control as unity—the ratio being called the Protection Factor (PF).

Thus

$$\text{PF} = \frac{\text{the stability of the sample containing antioxidant}}{\text{the stability of the control sample}}$$

The latter means of expressing results would have an advantage if the protection factor for a given antioxidant were reproducible even in different samples of the same type of fat. The factor, for example, will generally be much higher in lard of low initial stability than in lard of high initial stability. Care should always be taken to include other pertinent information such as the concentration of the antioxidant and the stability of the controls. Since the protection factors of an antioxidant will also vary depending on the test used, the concentration of antioxidant, and the control substrate, comparisons of different antioxidants at best should be limited to a series where these factors are constant.

The accelerated stability tests have also been used to determine the effect of proöxidants, such as traces of metal salts, and of metal deactivators. Further discussion of metal deactivators and synergists will be given later in the chapter.

In general, there is very poor correlation between the effectiveness of antioxidants in dry whole-fat substrates and the protection afforded foods made with these same stabilized fats. Antioxidants which may stabilize lard against rancidity for over a year at room temperatures, may be almost worthless in ham and many meat products. Therefore, an antioxidant must finally be evaluated in each particular food product.

Antioxidants

Chance observations of retardation of autoxidation were recorded as early as about a century ago. According to Bailey[17], who reviewed the literature up to 1937, one of the earliest references is that of Chevreul[54] in 1856. Chevreul reported that a film of linseed oil required more time to dry on oak than on poplar or pine wood, and less time on glass than on either of the woods. This retarding effect was attributed later[223] to the antioxygenic action of tannin in the wood, oak bark being rich in this phenolic substance. The first comprehensive search for substances which would inhibit autoxidation was undertaken during World War I by Moureu and Dufraisse. The incentive was to stabilize acrolein against oxidation and polymerization. Acrolein at that time was considered of importance in chemical warfare. After many empirical tests they found that traces of hydroquinone or pyrogallol maintained the acrolein clear and unaltered, and permitted large-scale production. This observation of the mysterious inhibiting effect

of phenols had a powerful catalytic effect on Moureu and Dufraisse, stirring up boundless enthusiasm and interest, which resulted in prodigious yields. (Bailey (*loc.cit.*) devoted a chapter to a review of their classical work.) In 1922[222, 221], they reported the following compounds as retarders of autoxidation and proposed the name "antioxygens": phenol, thymol, guaiacol, eugenol, α- and β-naphthol, hydroquinone, catechol, resorcinol, pyrogallol, gallic acid, tannin, salicyl aldehyde, vanillin, *o*-nitrophenol, and *o*-aminophenol. The autoxidizable substrates in which these compounds were tested included benzaldehyde, acetaldehyde, chloral, propyl aldehyde, acrolein, anisaldehyde, furfural, styrolene, terpentine, linseed oil, walnut oil, and butter. They noted also that the most effective phenols had at least two hydroxyls on the benzene nucleus in ortho- or para-position, and that α-naphthol was much superior to β-naphthol. Later they found[224] also that secondary amines, diamines, and aminophenols were very effective antioxidants, while pyridine, quinoline, amides, and nitroso-bases were of little value. Over 500 different compounds and substances were tested by Moureu, Dufraisse and co-workers in about 100,000 separate oxygen absorption experiments[17]. Their theory on mode of action of antioxidants will not be reviewed except to mention that it was based on marked power of selective attraction of antioxidants for activated or oxygenated molecules of autoxidizable substrate, and that antioxidants must reduce peroxides. Within a few years others had made notable studies on the autoxidation of linseed oil and unsaturated organic compounds as affected by catalysts and by antioxidants of the phenolic and aromatic amine type[270, 271, 295, 315, 331, 280].

Following this phenomenally rapid development in which nearly every conceivable type of compound had already been tested, attention was directed toward possible application of antioxidants in foods to prevent rancidity. Most of the previous work had been done on nonedible products other than fats. Furthermore, little was known of the pharmacology of the many phenolic and amine compounds, although some were suspected of being toxic. Early studies on autoxidation of various vegetable oils and animal fats showed significant differences in induction periods which could not be explained by differences in unsaturation. It was suspected that the presence of natural antioxidants accounted for the longer induction periods. Therefore, extensive efforts were directed toward the isolation and identification of natural antioxidants preferably from edible sources on the reasonable assumption that the addition of such substances to unstable fats and foods might be permitted. Lundberg[186] reviewed the literature on antioxidants up to 1947, and the writer is grateful for help received from his review.

Principal attention will be given to antioxidants which have been investigated thoroughly and are authorized for addition to animal fats, and to those which have received serious consideration for use in foods. The requirements of an ideal antioxidant have been stated[143]: it should have no harmful physiological effect nor contribute objectionable flavor, odor, or color to the fat or to foods made with the fat; be effective in retarding rancidity and the effectiveness should carry through to foods made with the fat; be sufficiently fat soluble so that it can be incorporated with ease; be readily available and inexpensive.

With appropriate label declarations[257] the following substances (preservatives) may be added, in the amounts indicated, to rendered animal fat or a combination of such fat and vegetable fat:

"(1) Resin guaiac not to exceed 1/10 of 1 per cent; or

"(2) Nordihydroguaiaretic acid not to exceed 1/100 of 1 per cent; or

"(3) Tocopherols not to exceed 3/100 of 1 per cent. (A 30 per cent concentration of tocopherols in vegetable oils shall be used when added as a preservative to products designated as "lard" or "rendered pork fat"); or

"(4) Lecithin: Provided, that nothing in this paragraph shall prevent the use of this substance as an emulsifier as approved by the chief of division; or

"(5) Citric acid not to exceed 1/100 of 1 per cent; or

"(6) Citric acid not to exceed 5/1000 of 1 per cent, or phosphoric acid not to exceed 5/1000 of 1 per cent, in combination with not more than 1/100 per cent of nordihydroguaiaretic acid; or

"(7) Propyl gallate not to exceed 1/100 of 1 per cent; or

"(8) Propyl gallate not to exceed 1/100 of 1 per cent in combination with not more than 5/1000 of 1 per cent of citric acid; or

"(9) Thiodipropionic acid, dilauryl thiodipropionate, distearyl thiodipropionate or combinations thereof in quantities not to exceed 1/100 of 1 per cent of thiodipropionic acid and 9/100 of 1 per cent of either dilauryl thiodipropionate or distearyl thiodipropionate or combinations of the two; or

"(10) Butylated hydroxyanisole (a mixture of 2-tertiarybutyl-4-hydroxyanisole and 3-tertiarybutyl-4-hydroxyanisole) and combinations of butylated hydroxyanisole with nordihydroguaiaretic acid or propyl gallate with or without the addition of citric acid or phosphoric acid, may be added as preservatives to animal fats and shortenings containing animal fats. The quantities used shall not exceed 2/100 of 1 per cent of butylated hydroxyanisole, or 1/100 of 1 per cent of nordihydroguaiaretic acid plus 2/100 of 1 per cent butylated hydroxyanisole or 1/100 of 1 per cent of propyl gallate plus 2/100 of 1 per cent of butylated hydroxyanisole. Citric acid or phosphoric acid, not to exceed 5/1000 of 1 per cent may be added with butylated hydroxyanisole or with the combinations of butylated hydroxyanisole and nordihydroguaiaretic acid or propyl gallate."

In addition to the above, and with appropriate label declarations mono-

isopropyl citrate may be used as follows[207]: "Monoisopropyl citrate may be added as a preservative to animal fats and shortenings containing animal fats in an amount not to exceed 1/100 of 1 per cent."

"When used with other approved fat preservatives, the amount of monoisopropyl citrate may not exceed 5/1000 of 1 per cent."

Lecithin and Related Substances. Lecithin was probably the first natural substance to receive consideration as an antioxidant for edible fats and oils. The term lecithin has been applied to commercial preparations of mixed phospholipids obtained chiefly as a by-product in the manufacture of soybean oil and corn oil. It is a complex mixture containing lecithin, cephalin, phosphoinositides, sugars, sterols, sterol glucosides, pigments, and antioxidants[337]. The antioxidants presumably are minor amounts of tocopherols. Woolley[339] isolated a fraction of phosphoinositides ("soybean lipositol") which had a molecular ratio of 1:1:2 for inositol, phosphoric acid, and fatty acids, and contained no glycerol. Scholfield *et al.*[283] reported a sample of oil-free phospholipids to contain 24 per cent lecithin, 25 per cent cephalin, and 33 per cent phosphoinositides.

The use of commercial lecithin was first proposed by Bollmann in 1923[43]. It is available in large quantities and at low cost. No question has been raised about the edibility of lecithin and it has been used in food products for many years—in some instances more because of its emulsifying properties. There appears to be some doubt that lecithin is a primary antioxidant; its effect as inhibitor being more like that of a synergist or metal deactivator[30]. The cephalin component of commercial lecithin appears to be responsible for most of the synergistic or inhibiting effect[240]. The effectiveness of lecithin including its similarity to the effect of phosphoric acid has been described[241, 15, 238, 244, 265, 268, 313, 322]. Examples of stabilities of fats obtained with added lecithin are in Tables 8-4 and 8-5.

Since phosphoric acid and some of its derivatives appear to be similar in action to that of lecithin, references to some of these compounds are included: phosphoric acid[15, 80, 117, 238, 261, 176]; phosphorus acid or salts[260, 67]; esters of phosphoric acid[84, 82, 274, 231, 197].

Tocopherols and Related Compounds. Concurrently with the development of lecithin as an inhibitor, Anderegg and Nelson[5] and Mattill[201] observed that wheat-germ oil added to experimental diets containing animal fats retarded oxidative rancidification. It was also observed that rancidity was always accompanied by destruction of vitamin E. It was concluded from their work, along with that of Evans and Burr[91] that animal fats contain little if any natural antioxidants whereas wheat-germ oil is relatively rich in such substances. Other evidence of natural antioxygens was soon reported: unsaponifiable fractions from corn-germ oil[203], lettuce lipides[242], cottonseed, wheat germ, and palm oils[241]. The close association in these

TABLE 8-4. STABILITY OF LARD, COTTONSEED OIL, AND HYDROGENATED COTTONSEED OIL CONTAINING ADDED ANTIOXIDANTS*

	Stability† (AOM)				
	Cottonseed oil		Hydrog. cotton seed oil	Lard	
Antioxidant concentration	0.1%	0.05%	0.01%	0.01%†	0.01%‡
	hr	*hr*	*hr*	*hr*	*hr*
Control (no antioxidant)	9.1	9.1	121	4.1	5.0
α-Tocopherol	7.6	7.1	107	17.0	18.5
γ-Tocopherol	9.0	8.3	135	19.3	30.0
Lecithin	10.6	11.3	172	4.8	5.5
α-Conidendrol	10.1	13.0	153	25.5	33.1
β-Conidendrol	11.4	17.5	174	32.5	44.0
Norconidendrin	14.1	22.1	152	25.8	31.0
Nordihydroguaiaretic acid	10.0	18.7	120	50.0	62.5
Gum Guaiac	6.6	8.5	100	3.4	4.5
Hydroquinone	9.3	35.0	128	65.0	70.0
Sesamol	8.8	15.5	98	30.8	38.5
Propyl gallate	15.8	54.9	172	43.8	47.5
Butylated hydroxyanisole	6.6	7.3	108	19.3	31.0
Di-tert-butyl-*p*-cresol	9.4	14.6	118	22.7	26.5

* Taken from published data[214].
† 20 Meq/kg used as end of induction period.
‡ 100 Meq/kg used as end of induction period.

oils of vitamin E and antioxygenic activity[71], the isolation of vitamin E (α-tocopherol)[92] and other tocopherols[82, 299], and the oxidation inhibiting effect of pure tocopherols[239], led to the conclusion that these oils owe their antioxygenic activity chiefly to these substances. Tocopherols have been reported in many seed oils, fruits, and vegetable and animal tissues. A recent review of their occurrence in natural sources has been published[172].

Tocopherol is the only antioxidant that is deposited in adipose tissues of animals to any appreciable extent[139]. It is the principal natural antioxidant of hog fats, being present in about 1 to 3 parts per 100,000[57], whereas the more common vegetable oils contain from about 30 to 100 parts per 100,000. A great deal of interest was created in the tocopherols because of their nutritional significance as well as antioxygenic activity. They have the further advantage of being readily fat soluble. Along with ascorbic acid or appropriate synergist they offer good protection to fats and oils. The protection, however, does not appear to carry-over well to baked crackers[143]. This is a little surprising in view of the fact that vegetable shortenings of comparable unsaturation and containing tocopherols generally present no serious stability problem in crackers. It suggests that further attention

TABLE 8-5. STABILITY OF LARD CONTAINING BHA AND COMBINATIONS OF BHA WITH SYNERGISTS*

Antioxidant (%)	AOM (hr)	Protection factor
None (control lard)	8	1.0
BHA 0.01	32	4.0
BHA 0.01 + methionine 0.01	38	4.8
BHA 0.01 + citric acid 0.005	39	4.9
BHA 0.01 + methionine 0.01 + citric acid 0.005	46	5.8
None (control lard)	2	1.0
BHA 0.01	16	8.0
BHA 0.01 + H_3PO_4 0.002	21	10.5
BHA 0.01 + triethyl phosphate 0.002	20	10.0
BHA 0.01 + ethyl acid phosphate 0.002	21	10.5
Lecithin 0.10	4	2.0
BHA 0.01 + lecithin 0.10	26	13.0
BHA 0.01 + lecithin 0.01	21	10.5
BHA 0.01 + citric acid 0.002	36	18.0

* Taken from published data[166]; BHA = butylated hydroxyanisole.

should be given to the use of slight hydrogenation to reduce the most highly unsaturated components of lard, together with the addition of tocopherols and synergists. The tocopherols, of course, need not be added as pure compounds. They may be added to meat fats simply by mixing tocopherol-bearing oils or these oils hydrogenated. Riemenschneider *et al.*[268] reported the addition of 1 to 10 per cent of certain vegetable oils or shortenings to lard increased the stability from 2 to 5-fold. Magoffin and Bentz[194] reported excellent results from an equal mixture of vegetable shortening and lard for frying potato chips; the chips kept about as well as those fried in vegetable shortening alone (see Table 8-10). Other work on this type of mixture has been published[264, 9, 182, 320] and patents have been issued[1, 128, 129, 208, 235].

The antioxygenic properties of pure tocopherols and concentrates rich in tocopherols have been extensively investigated[114, 16, 135, 212, 244, 265, 291, 313, 132], and patents on their use have been granted[8, 23, 79, 319]. Typical data on their effectiveness are included in Tables 8-2, 8-3, and 8-4.

Compounds related to the tocopherols, such as hydroxy chromans, and hydroxy coumarans have also been reported effective inhibitors of rancidity[115, 204, 294, 112].

Gum Guaiac. Gum guaiac, a resinous secretion of a tropical evergreen tree, *Guaiacum officinalis*, indigenous to the West Indies, was first proposed as an antioxidant for fats by Newton and Grettie[234] and described by Grettie[127]. The guaiac resin contains complex phenolic constituents related chemically to guaiacol, guaiaretic acid, and guaiaconic acid. Presumably it owes its antioxygenic properties to one or more of these phenols. The resin also contains significant amounts of inactive insoluble material. The antioxidant may be added to the fat during rendering, the insoluble matter and foreign odor being removed by subsequent filtration and deodorization. Another method is to extract the active constituents of the resin with a solvent such as acetic acid[74]; the filtered extract is then added to the fat at a fairly high temperature and the solvent removed by deodorization. Like other phenolic antioxidants, gum guaiac is more effective in animal fats than in vegetable oils, but unlike many, its protection carries over reasonably well into crackers made with the stabilized fat[143, 127, 212]. After extensive pharmacological investigations and proof of its innocuous nature was established, gum guaiac was authorized for use in lard and rendered pork fat. This antioxidant was the first substance from a non-food origin permitted as a preservative for lard and rendered pork fats. Data on its effectiveness are included in Tables 8-2, 8-3, and 8-4.

Nordihydroguaiaretic acid (NDGA) and Related Substances. Like gum guaiac, NDGA is a phenolic substance obtained from a natural non-food source. It is obtained by extraction[110] from the desert plant, *Larrea divaricata*, one of several species of plants commonly known as creosote bush, which is a common plant in the desert areas of our southwestern states. According to Lundberg *et al.*[190, 186], who first described its antioxidant properties, NDGA is only sparingly soluble in fats, about 0.5 per cent at 45°C[189]. It may be incorporated into fats by the use of a solvent as described for gum guaiac, or by making a 0.5 per cent solution in hot fat and adding appropriate amounts of the solution to the main portion of fat.

Chemically, NDGA is related to catechol and may be considered as an alkyl substituted catechol. Data on the effectiveness of this compound are included in Tables 8-2, 8-3, 8-4, and 8-6, along with data showing the increased stability when synergists are also added. NDGA is a very effective antioxidant for lard and the protection carries through to some extent to baked crackers (see Table 8-3). Like most phenolic antioxidants, however, it is not particularly effective in cottonseed or soybean oils. In storage tests at room temperature lard stabilized with NDGA remained organoleptically sweet for more than a year[190].

NDGA is physiologically harmless at the low concentrations in which it is used, and authorization has been granted for its addition to lard and rendered pork fat. A public service patent by Lauer[174] covers its use as an antioxidant.

Numerous investigators have evaluated NDGA in different fats and foods by one or more accelerated tests[9, 24, 31, 30, 199, 266, 214, 169, 180, 326, 278, 277, 276, 59, 163, 33]. In most instances, comparative results were also shown for other phenolic inhibitors and with different synergists or metal deactivators. Compounds related to NDGA in structure have been synthesized by Gisvold[111].

TABLE 8-6. STABILITY OF LARD CONTAINING ANTIOXIDANTS AND SYNERGISTS*

Antioxidant (%)	Stability AOM	
	Stabilized	Control
	hr	*hr*
NDGA 0.001	15	6
NDGA 0.001 + citric acid 0.005	26	6
NDGA 0.01	40	4
NDGA 0.01 + citric acid 0.01	119	4
NDGA 0.005	58	7
NDGA 0.005 + citric acid 0.01	67	7
NDGA 0.005 + d-isoascorbyl palmitate 0.005	65	8
Propyl gallate 0.005	40	7
Propyl gallate 0.005 + citric acid 0.01	50	7
Catechol 0.01	44	7
Catechol 0.01 + citric acid 0.01	48	7
Gallic acid 0.005	33	8
Hexyl gallate 0.005†	55	8
Lauryl gallate 0.005†	42	8
Octadecyl gallate 0.005†	55	8

* Taken from published data [186, 266].
† Molecular equivalent of 0.005% gallic acid[216].

Norconidendrin, and α- and β-conidendrols, are included here because they have some similarity in structure to NDGA. This similarity was pointed out by Fisher *et al.*[104], who first prepared norconidendrin from conidendrin and described its antioxygenic properties. Norconidendrin and the conidendrols presumably are isomeric demethylation products, although the exact relationship to α-norconidendrin and β-norconidendrin (described by Hearon *et al.*[141, 140] has not been made clear.

Conidendrin, the parent substance, has been isolated from a number of coniferous woods. Its recovery as a by-product from the sulfite waste liquors produced in the pulping of western hemlock would provide large quantities in relatively pure form[44, 248].

Norconidendrin and α- and β-conidendrols have been evaluated in a comparative way with a number of other well-known antioxidants for lard, cottonseed oil, and hydrogenated cottonseed oil[214], for candies[105], and for GR-S polymers[191]. Its effectiveness in lard was not greatly different from that of NDGA when compared at 0.05 per cent concentration by the AOM; at the more "realistic" level of 0.01 per cent, however, the stability values with NDGA were significantly greater. Of the antioxidants tested propyl gallate appeared to be superior for both cottonseed oil and hydrogenated cottonseed oil, and one of the best for lard. Some of the results are shown in Table

Propyl Gallate and Related Substances. Propyl gallate was first proposed as an antioxidant for fats by Sabalitschka and Boehm[275]. Boehm and Williams[37, 36] reported it to be highly effective in fats and oils. Lea[175] showed that the methyl and ethyl gallates were also highly effective antioxidants.

These lower esters of gallic acid are readily prepared by ordinary esterification procedures using mineral acid catalyst and are low in cost. They are sparingly soluble in fats and oils and appreciably soluble in water. Higgins and Black[143] showed that propyl gallate was highly effective in stabilizing lard and also was effective, but to a lesser extent, in cottonseed and soybean oils. The protection, however, did not carry over to baked crackers. Morris and Riemenschneider[218] prepared higher alkyl gallates to determine the effect of greater fat solubility on the antioxygenic properties. The higher esters were readily soluble in fats and were somewhat more effective than propyl gallate in lard when the antioxidants were added in molecular equivalent amounts, and were also slightly superior in piecrust but neither ester was effective in crackers[219, 216]. Typical data obtained in the evaluation of propyl gallate and other gallates and related compounds are presented in Tables 8-2, 8-3, 8-4 and 8-6.

In common with most of the phenolic antioxidants, the gallates appear to be most effective when a synergist is also added. Propyl gallate is the only ester of gallic acid which has been given specific authorization for use in meat fats although there is little reason to believe that other alkyl esters would have a different physiological effect. In recent extensive pharmacological tests the evidence indicated that no harmful effects would be produced by ingestion of amounts likely to be encountered in edible fats and oils protected against oxidation[3, 329].

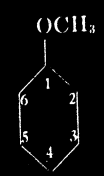
Gallic acid, the compound from which the esters are derived, occurs naturally in teas, and as gallo-tannins and glucosides in nut galls and natural tannins. It is inexpensive and effective for both animal and vegetable fats. The carboxyl group of gallic acid has been reported to act synergistically with the antioxygenic hydroxyls of this compound[118]. Other workers have supplemented our knowledge of the antioxygenic properties of gallic acid and its esters[102, 199, 189, 276, 214, 277, 180, 169, 195, 245]. In many references cited, results with antioxidants and synergists were reported. Certain synergistic mixtures have been patented[136, 137].

Tannins, a natural source from which gallic acid is obtained by hydrolysis, have been studied as a means of stabilization of fats[69, 296, 230, 330]. The tannin may be added to the lard at about 70 to 75°C with stirring and then filtered. The tannin appears to act as a scavenger for trace metal impurities[296].

Butylated Hydroxyanisole and Related Compounds. The first

publications describing the antioxygenic activity of butylated hydroxyanisole (BHA) in fats in a comprehensive way were those of Kraybill *et al.*[165, 166, 167]. BHA and certain combinations of BHA with hydroquinone, propyl gallate, acid type synergists (i.e., citric and phosphoric acids), lecithin, methionine, and thiodipropionic acid were very effective in animal fats, and in crackers, pastry and potato chips made with the fat. According to Kraybill *et al.*, commercial BHA consists chiefly of two isomers: 3-tertiarybutyl-4-hydroxyanisole and 2-tertiarybutyl-4-hydroxyanisole. It is readily soluble in fats and oils but nearly insoluble in water, is nontoxic, and imparts no detectable odors to the product.

Rosenwald and Chenicek, who earlier had reported the effectiveness of BHA as an antioxidant for gasoline[272], also recently published on the effectiveness in lard of the two isomers of BHA and of a series of derivatives of anisole[273]. Their data indicated the importance of the position of the hydroxyl and alkyl substituents in the benzene nucleus in relation to the methoxyl group. If the hydroxyl is adjacent to the methoxyl or in the 3-



anisole

position the compound is ineffective, whereas 4-hydroxyanisole has significant activity. If the nucleus is further substituted with alkyl groups, the size and structure of the group as well as the position in which it is substituted has an important influence on antioxygenic activity (see Table 8-7). Thus, the 2-methyl derivative of 4-hydroxyanisole is not as potent as the 3-methyl derivative. The influence of size and structure of the alkyl group is indicated by the finding that 3-tertiarybutyl-4-hydroxyanisole is more potent than the 3-methyl or 3-*n*-butyl derivatives of 4-hydroxyanisole. The 3-tertiarybutyl isomer was 2 to 4 times more effective in a sample of lard than the 2-position isomer and about 2½ times more effective in crackers.

Dugan *et al.*[76] also observed a significant difference in activity between the two isomers. The 3-tertiarybutyl derivative, however, was only about 1½ times as effective as the 2-isomer in 11 different samples of lard and only slightly better in carry-over in crackers. They found no decided advantage of the 3-isomer over the commercial BHA, a mixture of the two isomers. Compounds with an alkyl substitution adjacent to the hydroxyl were classed as "partially hindered" phenols.

BHA alone and in combination with propyl gallate and citric acid retarded the development of rancidity in corn and peanut oils during deep-

TABLE 8-7. STABILITY OF LARD CONTAINING DERIVATIVES OF HYDROXYANISOLES*

Compound	AOM stability	
	0.005%†	0.02%†
	hr	*hr*
None (control lard)	(3)‡	(3)‡
4-Hydroxyanisole	7.5	13.5
2-Methyl-4-hydroxyanisole	6	17
3-Methyl-4-hydroxyanisole	12.5	19
3,5-Dimethyl-4-hydroxyanisole	12	15
2,5-Dimethyl-4-hydroxyanisole	13	17
2,6-Dimethyl-4-hydroxyanisole	<4	<5
3-*n*-Propyl-4-hydroxyanisole	12.5	15.5
3-Allyl-4-hydroxyanisole	10	15.5
3-Isopropyl-4-hydroxyanisole	10	16
Di-isopropyl-4-hydroxyanisole	11	15
3-*n*-Butyl-4-hydroxyanisole	12	17.5
3-Isobutyl-4-hydroxyanisole	11.5	16
3-*sec*-Butyl-4-hydroxyanisole	14	20.5
3-*t*-Butyl-4-hydroxyanisole	21	32
2-*t*-Butyl-4-hydroxyanisole	6	13.5
2,5-Di-*t*-butyl-4-hydroxyanisole	12	17
3-Crotyl-4-hydroxyanisole	9	15.5
3-β-Methallyl-4-hydroxyanisole	10.5	16
3-α-Methallyl-4-hydroxyanisole	11	18
3-*t*-Butyl-5-methyl-4-hydroxyanisole	21	35
5-*t*-Butyl-2-methyl-4-hydroxyanisole	18	24
2-*t*-Butyl-5-methyl-4-hydroxyanisole	10	13.5
Hydroquinone	40	93.5
Mono-*t*-butylhydroquinone	24	80
2,5-Di-*t*-butylhydroquinone	6	14
t-Butyl-1,4-dimethoxybenzene	—	3
3-Hydroxyanisole	<4	3
4-*t*-Butyl-3-hydroxyanisole	<4	7
4,6-Di-*t*-butyl-3-hydroxyanisole	<5	7
Resorcinol	3	<4
2-Hydroxyanisole (guaiacol)	<4	<4
3-*t*-Butyl-2-hydroxyanisole	<5	<5
4-*t*-Butyl-2-hydroxyanisole	<5	<5
5-*t*-Butyl-2-hydroxyanisole	<5	<5
5-Methyl-2-hydroxyanisole (creosol)	—	<4
3-*t*-Butyl-5-methyl-2-hydroxyanisole	<5	7
4-*t*-Butyl-5-methyl-2-hydroxyanisole	<4	7
Catechol	25	55
t-Butylcatechol	37	65.5

* Taken from published data[273].
† Concentration of compound added.
‡ No compound added.

fat frying, and in foods fried in these oils[77]. It also was effective in delaying rancidity and vitamin A destruction in mixed feeds, such as dry dog meal. The antioxidant has also found application in stabilizing nuts, chicken fat, pastry, and in oils and waxes used to treat papers for food wrappers.

Other workers have substantiated the general effectiveness of BHA and combinations with synergists, such as have been authorized for use in animal fats, and have extended our information on its antioxygenic action[25, 153, 195, 52, 276, 326, 180, 214, 169, 164, 192]. As a result of the general effectiveness of this substance in different fatty foods and how well it approaches the requirements of an ideal antioxidant, it has become the most widely used antioxidant to date. Typical data obtained in the evaluation of BHA and certain synergistic combinations are included in Tables 8-5, 8-8, 8-9, and 8-10.

Another compound of the "hindered" phenolic type which is receiving careful consideration is 2,6-di-tertiarybutyl-4-methylphenol (2,6-di-tertiarybutyl paracresol). In analogy to butylated hydroxyanisole this compound could be referred to as butylated hydroxy toluene.* This compound probably first received attention for use in gasoline and petroleum products[300, 301, 304]. Consideration of this material for use in edible fats and oils is relatively recent and very little has been published on it. Moore and Bickford[214] included this compound in a series of others for comparative evaluation by the AOM in lard, cottonseed oil, and hydrogenated cottonseed oil (see Table 8-4). Likewise, Kring[169] made comparative tests with this compound, alkyl esters of gallic acid, hydroquinone, NDGA, and BHA in the following substrates: lard, peanut oil, soybean oil, codliver oil, and peanut oil containing added vitamin A. The samples were stored at 35°C. All the compounds had noticeable antioxygenic effect but the lower esters of gallic acid seemed to be superior. Citric and phosphoric acid generally enhanced the effect.

Thiodipropionic Acid and its Dialkyl Esters. The only nonphenolic compound, which appears to be a primary antioxidant for fats and oils and which has received authorization for addition to meat fats, is thiodipropionic acid and its lauryl and stearyl esters. The writer was unable to find much published information on the antioxygenic properties of this substance, other than that disclosed in patents[243, 131] on this and related compounds and several incidental AOM stability tests[166] showing its effect in lard. From the limited published information, the acid, esters, or combination of the two are effective antioxidants in fats and oils and rapidly increase in effectiveness with increase in concentration. Remarkable "synergism" was noted when the acid was added to lard along with BHA[166] (see Table 8-8).

Other Antioxidants. Little useful purpose would be served in attempting to describe numerous published works in which such well-known antioxidants as hydroquinone, catechol, and pyrogallol have been evaluated. In many of the references cited, these compounds have been included for comparative purposes, and examples are also included in some of the tables here for the same reason.

* Since this manuscript was completed, butylated hydroxytoluene (2,6 di-tertiary butyl paracresol) has also been made permissive, effective June 30, 1954, as a preservative for rendered animal fats and shortenings containing rendered animal fats in amounts not to exceed 1/100 of 1 per cent.

TABLE 8-8. STABILITY OF LARD CONTAINING BUTYLATED HYDROXYANISOLE, OTHER ANTIOXIDANTS, AND SYNERGISTS*

Antioxidant or synergist (%)	AOM (hr)	Protection factor
None (control lard)	2	1.0
H_3PO_4 0.005	2	1.0
BHA 0.01	38	19.0
TDPA 0.01	19	9.5
BHA 0.01 + TDPA 0.01	81	40.5
TDPA 0.01 + H_3PO_4 0.005	4	2.0
BHA 0.01 + TDPA 0.01 + H_3PO_4 0.005	65	32.5
BHA 0.01 + H_3PO_4 0.005	45	22.5
None (control lard)	4	1.0
BHA 0.01	21	5.3
PG 0.003	26	6.5
Hq 0.003	27	6.8
BHA 0.01 + PG 0.003 + CA 0.002	60	15.0
BHA 0.01 + Hq 0.003 + CA 0.002	59	14.8

* Taken from published data[166]; BHA = butylated hydroxyanisole; TDPA = thiodipropionic acid; PG = propyl gallate; Hq = hydroquinone; CA = citric acid.

TABLE 8-9. STABILITY OF CRACKERS, PASTRY AND POTATO CHIPS MADE FROM LARD CONTAINING ISOMERS AND MIXTURES OF ISOMERS OF BHA*

Antioxidant†	Schaal oven stability—145°F		
	Crackers‡	Pastry‡	Potato chips‡
	hr	*hr*	*hr*
None (control lard)	161	135	98
3-isomer BHA	777	1166	1003
75% 3-isomer, 25% 2-isomer BHA	707	1157	924
50% 3-isomer, 50% 2-isomer BHA	670	1101	778
25% 3-isomer, 75% 2-isomer BHA	697	1115	755
2-isomer BHA	633	1107	759
Commercial BHA	743	1071	795

* Taken from published data[76].
† Total antioxidant concentration in each test 0.01%.
‡ Average value from 4 lards.

Likewise, there are many substances which have received only superficial examination or very little publicity to date, and others which have not found wide application for unknown reasons. A partial list of these substances is as follows:

1,5-dihydroxynaphthalene[175]
condensation product of polyphenol with a ketone[175, 209]
hematoxylin[175]
sesamol[17, 46]
caffeic or hydrocaffeic acid[81, 205]
2,5-dihydroxyphenyl dimethylcarbinol[26]
β-thioketo sulfoxides and sulfones[321]
antioxidants of natural spices[56]
crude source of vitamin B-complex[133] and hydroxyanisole[131]
nuclear-substituted cinnamyl alcohol[2]
phenylhendecapentenal and related aldehydes[338]
antioxidants of peel, pulp, or albedo tissue of citrus fruits[250]
p-amino benzoic acid[237, 236]
5-pentadecylresorcinol[20]
antioxidants from rice bran[216, 217, 153]
antioxidant of osage orange[60]
derivatives of *p*-phenylenediamine[64]
derivatives of quinoline[199]
isoeugenol or diisoeugenol[152]
antioxidants of anise seed[155]
extracts from cereal grain[66, 151, 229, 72, 184, 227, 219]
Southern sweet gum[206]
alcohol extract of blackstrap molasses[228]
gossypol[202, 85, 29]
thyroxine[73]
quercitin or dihydroquercitin[121, 171]

Synergists or Metal Deactivators. The effect of acidic compounds such as ascorbic, citric, tartaric, and phosphoric acids has been mentioned earlier and examples of their enhancing effect on the stability of fats in the presence of primary antioxidants of the phenolic type have been included in tabular data. In the earlier work reported, this enhancing effect was attributed to a synergistic action and the term synergist for these compounds continues to be used, even though their function as metal deactivators has become known, probably because no one has proved conclusively that the effect is entirely attributable to metal deactivation. The suggestion that these compounds function as metal deactivators in fats is not particularly new[90, 263, 32]. Morris *et al.*[217], presented evidence which stressed the influence of trace metals and the importance of these acidic compounds in connection with the use of primary antioxidants in the presence of trace metals. Some of their results are reproduced in Tables 8-11, 8-12, 8-13, and 8-14. Ascorbic acid, ascorbyl palmitate and potassium ascorbyl palmitate were

TABLE 8-10. KEEPING QUALITY OF POTATO CHIPS FRIED IN DIFFERENT FATS WITH AND WITHOUT ADDED ANTIOXIDANTS*

Test No.†		Keeping quality of chips at 145°F (days)
1	Lard (control)	5
2	Lard + BHA‡	28
3	Lard + BHA combination§	32
4	Peanut oil (control)	4
5	Peanut oil + BHA	13
6	Peanut oil + BHA combination	14
7	Cottonseed oil (control)	4
8	Cottonseed oil + BHA	6
9	Cottonseed oil + BHA combination	7
10	Vegetable shortening (control)	25–30
11	Vegetable shortening + BHA	47
12	Vegetable shortening + BHA combination	58
13	Lard 50% + peanut oil 50% (control)	14
14	Lard 50% + peanut oil 50% + BHA combination	19
15	Lard 50% + vegetable shortening 50% (control)	20–30
16	Lard 50% + vegetable shortening 50% + BHA	47
17	Lard 50% + vegetable shortening 50% + BHA combination	47
18	Lard 40% + vegetable shortening 60% (control)	42
19	Lard 40% + vegetable shortening 60% + BHA	61
20	Lard 40% + vegetable shortening 60% + BHA combination	70
21	Vegetable shortening (control)	44
22	Vegetable shortening + BHA	65

* Taken from published data[191].
† Tests No. 1–17, laboratory scale fryings; 18–22, commercial fryings.
‡ 0.02% butylated hydroxyanisole added to frying fat.
§ 0.02% butylated hydroxyanisole, 0.006% propyl gallate, and 0.004% citric acid = BHA combination added to frying fat.

most effective in the presence of copper. The latter compound was effective against copper, iron and nickel as was also citric acid. Phosphoric acid offered protection to lard containing copper, iron, and nickel salts. When lauryl gallate was added to lard, however, phosphoric acid was not effective in the presence of iron salts. Compounds other than those listed in the text or tables enhance the stability of fats: ethylene diaminetetraacetic acid[285]; various amino acids[185, 59]; polyphosphates[332]; mono and dialkyl (or alkylene) esters of citric acid[123].

Trace metals appear to be an important contributing factor in promoting the first (preranced) oxidative changes which are characterized by "reverted" flavors. The tendency of oils and fats to revert is also associated with the presence of linolenic acid or possibly acids of higher unsaturation. Extensive investigations dealing with the problem of reversion in vegetable

TABLE 8-11. EFFECT OF METALS ON THE STABILITY (AOM) OF LARD[217]

Metals	Conc. (ppm)	Stability (hr)
Control (lard A)	—	7.0
Cu	10.0	0.3
Cu	5.0	0.3
Cu	1.0	0.4
Cu	0.5	0.5
Cu	0.4	0.5
Cu	0.3	0.5
Cu	0.2	0.8
Cu	0.1	3.2
Fe	20.0	0.3
Fe	10.0	0.3
Fe	5	0.3
Fe	3	0.4
Fe	2	0.5
Fe	1	2.0
Ni	2	1.0
Control (lard B)	—	6.0
Ni	4	1.0
Ni	10	1.0
Ni	20	1.0
Sn	10	3.0
Sn	20	1.0

TABLE 8-12. EFFECT OF METALS ON STABILITY OF LARD IN THE PRESENCE OF ANTIOXIDANTS[217]

Antioxidant	AOM stability in presence of				
	0.2 ppm Cu	2.0 ppm Fe	4.0 ppm Ni	20 ppm Sn	No metal
	hr	*hr*	*hr*	*hr*	*hr*
0.01% lauryl gallate	6.5	0.7	21	—	51
0.02% lauryl gallate	13	—	—	—	—
0.01% ethyl pyrogallol	35	1	72	18	126
0.02% ethyl pyrogallol	54	—	—	—	—
0.01% butylhydroxyanisole	7.5	4.2	15	19	22
0.01% mono- or diethyl ether of ethyl pyrogallol	1	1	3	4.5	12
0.01% ditertiary butyl-*p*-cresol	5.5	4.7	8.5	12	25
0.02% ditertiary butyl-*p*-cresol	9.5	—	—	—	39
0.01% β,3,4-dihydroxy-phenyl alanine	26	0.4	1.2	2	73
0.01% adrenalin	26	8	—	—	83
0.01% phloramine	11	0.7	11	8	35
0.01% methyl phloramine	21	1.5	26	13	54
0.01% JZF*	3.2	0.7	—	—	74

* Chiefly N,N′-diphenyl-*p*-phenylenediamine.

TABLE 8-13. EVALUATION OF SOME SYNERGISTS AS METAL DEACTIVATORS[217]

Synergist	AOM stability in presence of				
	0.2 ppm Cu	0.4 ppm Cu	2.0 ppm Fe	2.0 ppm Ni	No metal
	hr	*hr*	*hr*	*hr*	*hr*
Control (lard)	0.8	0.5	0.5	1.0	7
0.01% ascorbic acid	10	10	0.5	—	10
0.02% 1-ascorbyl palmitate	8	—	0.3	—	13
0.024% potassium 1-ascorbyl palmitate	11	—	8.5	10	14
0.01% tartaric acid	4	—	3	6	7
0.016% potassium tartrate	0.4	—	0.3	—	6
0.012% K-acid tartrate	0.4	—	0.3	—	7
0.01% citric acid	2.2	1.7	4.5	3.5	7
0.01% phosphoric acid	1.7	—	3.5	4.7	4.7
0.01% sorbitol	0.5	—	0.7	—	7
0.01% mannitol	0.4	—	0.3	—	3.2
0.01% lactic acid	0.4	—	0.5	—	6

TABLE 8-14. EVALUATION OF SYNERGISTS AS METAL DEACTIVATORS IN THE PRESENCE OF 0.01 PER CENT LAURYL GALLATE IN LARD[217]

Synergist	AOM stability in presence of 0.02% lauryl gallate and				
	0.2 ppm Cu	0.4 ppm Cu	2.0 ppm Fe	2.0 ppm Ni	No metal
	hr	*hr*	*hr*	*hr*	*hr*
None	6.5	—	0.7	21	51
0.01% ascorbic acid	60	59	0.7	41	63
0.01% 1-ascorbyl palmitate	67	62	0.3	50	68
0.024% potassium 1-ascorbyl palmitate	71	—	49	69	74
0.01% citric acid	33	26	40	49	56
0.01% phosphoric acid	29	—	1.5	31	59

oils, particularly soybean oil, have been under way for a number of years. A recent review of this work has been published by Dutton, Evans and Cowan[78] with particular emphasis on the present status of the problem, and including a comprehensive bibliography of the pertinent literature on the subject. The subject of reversion in fats and oils is only mentioned here chiefly to point out its close relation to the problem of rancidity. Trace metals are powerful promoters of both types of oxidative deterioration, and metal deactivators play an important part in the practical solution of both problems. In addition to the synergists or metal deactivators already mentioned in connection with rancidity the following compounds were also found effective in counteracting the promoting effect of metals in reversion:

phytic acid[86, 89]; chelating compounds containing nitrogen as coordinating atom: ethylendiamine tetraacetic acid, oxalohydramic acid, chelidamic acid, and α,α'-amino (acetic acid) propionic acid[284]; β-stearoxy-, and β-palmitoxy tricarballylic acids[285]; oxydiacetic and oxydipropionic acids[87]; thiodiacetic and β,β'-thiodipropionic acids, carbomethylmercaptosuccinic acid and its octadecyl ester[286, 88].

Antioxidants in Food Products

Much of the early work on retardation of spoilage of fats in food products was concerned with studies of factors which influence enzymatic changes as well as those which influence autoxidation. Similar oxidation products are produced through the action of lipoxidase[7]. The successful application in fats and oils of new and approved antioxidants within the past 10 to 15 years naturally stimulated a great deal of interest in their possible application to many fat-containing foods.

The problem of evaluating antioxidants in such products as meats, dairy products, fish, cereals, etc., is many times more difficult than in simple fats and oils because of the unpredictable influence of food components other than fats and including water and mineral salts. In some products, the fat is only a minor component. Hence the incorporation of antioxidants, satisfactory tests for stability, and the interpretation of results present many difficult problems. Although progress is being made in some products, it is understandable that the results in many cases are inconclusive and in other instances rather disappointing.

Attempts have been made to design stability tests for aqueous-fat systems which may give a better indication of the value of antioxidants for foods than tests conducted on dry fat substrates. In such artificial aqueous-fat substrates, polyphosphates and ascorbic acid were good synergists with phenolic antioxidants[275, 333, 332, 180]. BHA was one of the best primary antioxidants. In oxygen absorption measurements of methyl linoleate in contact with aqueous phases at controlled pH, the antioxidant efficiency of hydroquinone and NDGA dimished gradually from pH 5.1 to pH 9.2[297].

Dairy Products

According to Chilson *et al.*[55], one of the most difficult problems in market milk is the control of the oxidized flavor which often develops in several days storage at about 35°F. This problem is more troublesome when the cows are on winter feed than when they are on spring pasture. This oxidized flavor development appears to be related to variations in content of copper. Ascorbic acid is rapidly oxidized in the presence of copper and the addition of both to milk hastens the development of off-flavor. The addition of 10 to 20 mg. of propyl gallate per liter of milk retarded this oxidation considerably even in the presence of added copper (0.5 ppm). Esters of gallic acid were also effective in dried whole milk (323) but discoloration may result if appreciable iron salts are present. NDGA also was noticeably effective in delaying this flavor development in whole milk even in the presence of added copper[307]. The effectivness of ascorbic acid in whole milk appears to depend on the amount of copper present; if very low in copper, ascorbic acid retards oxidation. Ascorbic acid retards oxidized flavor in stored frozen milk[11, 6].

The application of antioxidants to dried milk has given more encouraging results. Phenolic antioxidants were effective particularly when used with synergists and added to milk before drying. Hollender and Tracy[145] reviewed the literature on this subject. Later work showed the following substances to be effective: wheat-germ oil[53], ethyl gallate and ascorbic acid[103, 179], NDGA and synergists[48], hydroquinone, cis-isosafroeugenol, ascorbic acid[232], tyrosine and its soluble esters, cereal flour and extract[65].

In butter oil, butter, and cream, the action of antioxidants is somewhat similar to that in lard in that the same antioxidants and synergists appear to be effective in retarding oxidation[255, 262, 226, 113, 21, 225, 251, 308, 325, 324, 27, 150, 288, 304, 292, 256, 306, 305]. Biacetyl was reported to be prooxygenic in butter and margarine[258]. It should not be inferred, however, that the use of antioxidants has been found entirely satisfactory. Often their use is accompanied by some reduction in flavor scores and in some cases slight discoloration.

Meat, Fish, and Poultry

The application of antioxidants to fresh meats, fish, and poultry has not proved satisfactory. Some progress is being made, however, and at least measurable beneficial effects have been reported in many studies. Antioxidants were ineffective in preventing rancidity in frozen herring[19]; ethyl gallate and ascorbic acid had noticeable preservation effect in storage of frozen salmon fillets[316, 317]; gallic acid, gum guaiac, ascorbic acid, and NDGA in cottonseed oil when used as a dip were effective to some extent in delaying rancidity of mackerel fillets stored in frozen condition[303]. A marked retarding of rancidity of turkey steaks in frozen storage was obtained by use of an aqueous gelatin coating containing either BHA, propyl gallate, or NGDA with citric acid[160]; the same antioxidants were effective in retarding rancidity during the cooking and subsequent frozen storage of turkeys[181]. Bentz *et al.*[28] discussed the general application of BHA in combination with propyl gallate and citric acid in stabilizing meats, fish, potato chips, crackers, pastry, butter, nuts, and in waxes used in coatings for foods. Phenolic antioxidants were found helpful in preventing color changes in meats[162].

Fish[18, 198, 289] and bacon[293, 161] dipped in aqueous solution of antioxidants and smoked were improved in keeping qualities. Volatile antioxygenic phenolic substances from the wood smoke penetrate the tissue and are about as effective as any means for delaying rancidity in this type of meat product.

Several general concluding statements are justified on the basis of progress made in the stabilization of fats and foods. With reasonable care in processing together with the judicious application of antioxidants and synergists (or metal deactivators), the problem of rancidity in fats, oils, and shortenings is well under control. A great deal more research is required before satisfactory solution to the problem of rancidity and oxidized flavors can be claimed in many fatty foods. Further progress in overcoming the problem in certain foods may depend on studies of the prooxygenic action of trace metals and of the role of synergists or metal deactivators. In some foods it appears that if the prooxygenic action of iron or copper could be effectively blocked, the addition of already known antioxidants would effectively delay rancidity. In this connection, further attention to avoidance of metal contamination in foods is of prime importance.

In view of the important role of linoleic acid (glycerides) in autoxidation of fats, perhaps greater emphasis should be placed on evaluation of antioxidants, proöxidants, and metal deactivators in pure methyl linoleate and aqueous emulsions of methyl linoleate. A few examples of effect of antioxidants in pure fatty acid ester substrates are included in Table 8-15.

The most effective antioxidants particularly from the standpoint of carry-over of protection to baked products and fried products such as potato chips are the "partially hindered" phenolics, typified by 3-tertiary-butyl-4-hydroxyanisole. Gallic acid, propyl gallate and possibly other esters of gallic acid are the most effective of the phenols for vegetable oils.

TABLE 8-15. EFFECT OF ANTIOXIDANTS AND SYNERGISTS ON THE OXYGEN ABSORPTION OF METHYL ESTERS OF FAT ACIDS. CONCENTRATION, .01%. TEMPERATURE, 100°C[302]

Antioxidant	Me linoleate		Me oleate		Distilled Me esters of lard	
	Stability*	Order of stability	Stability	Order of stability	Stability	Order of stability
	min		*hr*		*hr*	
None	11	10	2	10	1	10
α-tocopherol	41	9	8.5	9	3.5	8
α-tocopherol + C.A.†	67	8	35.0	5	8.5	5
α-tocopherol + soya lecithin + d-isoascorbyl palmitate‡	78	7	36.0	4	4.5	7
NDGA§	141	3	43.5	3	9.5	3
NDGA + C.A.	210	1	135.0	1	31.3	1
Propyl gallate	96	5	34.2	6	8.7	4
Propyl gallate + C.A.	158	2	101.0	2	25.4	2
Benzylhydroquinone	85	6	13.0	8	3.0	9
Benzylhydroquinone + C.A.	126	4	22.5	7	5.2	6

* Time required to absorb 1 g of oxygen per kg.
† Citric acid.
‡ Concentration, .02%.
§ Nordihydroguaiaretic acid.

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9. THE ESSENTIAL NUTRIENTS*

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The essential nutrients are those materials which must be supplied by the diet to produce and maintain optimum health. Several species of animals have now been raised to maturity upon diets of essentially known composition and there is reason to believe that practically all of the essential nutrients have been identified. Nearly all of these have been synthesized and the gross effects of the corresponding deficiency diseases have been documented. On the other hand, the absolute amounts required under varied conditions and by different species, the long term effects of slight deficiencies or excesses and the biochemical mechanisms involved, are still being actively investigated. Few statements in this field can be accepted as definite and the reader should be duly cautious. The original expectation that once the various factors were identified and requirements determined it would be relatively simple to determine which diets were adequate or inadequate, is still far in the future. We now recognize numerous interrelationships between nutrients, variation between individuals of the same species, great differences among species, intestinal synthesis of variable amount depending upon the diet and sanitary conditions, adaptation to diets, and there are undoubtedly other factors influencing requirements[1]. When one considers the permutations possible among the forty odd nutrients, the possibilities and difficulties become clear.

Nutritional requirements must be considered as approximations based upon the average individual and perhaps under average conditions as we understand them today. They are much less applicable to the individual. Recommended allowances as distinct from requirements are always set somewhat above estimated requirements to provide a margin of safety.

* See Section in Appendix on Recommended Dietary Allowances; also Chapters 12, 15, 17, 18, 19 and 20.