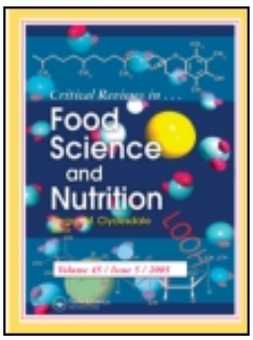

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KINETICS OF LIPID OXIDATION IN FOODS

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INTRODUCTION

Since 1961 when the Symposium on Foods: Lipids and Their Oxidation[1] was held, a great deal has been published on the development of rancidity in food stuffs. Unfortunately many of the questions asked at the close of the symposium have not been answered, nor the recommendations for future work heeded. This is especially true in the study of rancidity kinetics in actual foods. Tappel[2] commented, "That the future of lipid peroxidation studies must lie in the use of . . . . information of modern orbital theory, and quantum mechanics." This has not been done extensively with foods. However, in pure systems some information has been developed to give an idea of the reaction rates in foods.

The purpose of this review is to bring together the important work in foods and kinetics of the ten years since the conference which covered reaction rates and mechanisms. Much has to be glossed over or left out because it has been covered extensively elsewhere or the state-of-the-art has not been able to bring everything together. Most of the work on flavor development, testing procedures, and fat composition will not be covered. The main attempt will be to try to piece together the overall reaction of rancidity from a kinetic standpoint, on the basis of the physical-chemical properties of the food itself. It would be hoped that through this, processors could have a better handle for forecasting the shelf life of their products, and scientists could have a useful tool to apply modern theory to the complex biological system of food.

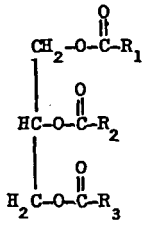
TYPES AND COMPOSITION OF LIPIDS IN FOODS

Much has been published on the lipid composition of foods, especially since the advent of the modern analytical techniques of IR, NMR, mass spectroscopy, and chromatography. It is well established that the fat content is not important, but rather the content of the fat, and its juxtaposition in the matrix making up the food. One can loosely define that there are depot fats for all foods such as the adipose tissue of animals, oil glands of various plants, and secondly interdispersed fats. These comprise free lipids that may be in the bloodstream or protoplasm of a cell, cell wall components, and bound lipids, such as the phospholipids found in mitochondria.

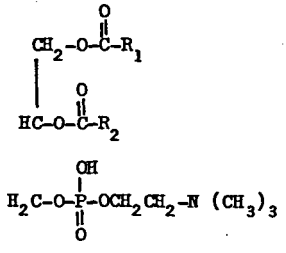
Most fats are in the form of triglycerides, many of which are of importance to rancidity. The triglyceride is a chemical moiety made up of glycerol to which on each hydroxyl group is attached via an ester linkage, a fatty acid (Table 1). The fatty acids are of primary importance to the rate of development of rancidity, since they can be very susceptible to attack by oxygen. Table 1 lists the major fatty acids found in foods and classifies them into a saturated or unsaturated category. It has been well established that the unsaturated fatty acid moieties cause the development of most rancid flavors in foods. The mechanism of the breakdown reaction has been reviewed extensively for oleate, linoleate, and linolenate[3-7] and will not be dealt with here. It should be noted, however, that it is not the total fat content that is important, but the amount of unsaturated fatty acid moieties. For example, beef contains a lot more fat than potatoes, but potatoes have a high polyunsaturated fat composition and are much more susceptible to oxidation when in the dehydrated state (Table 2). Values of known mixtures of pure fats submitted to various laboratories can vary by ± 20%, so these values may be subject to error. This error would be compounded in the procedures used to extract fat from a tissue.

TABLE 1
Lipid Components

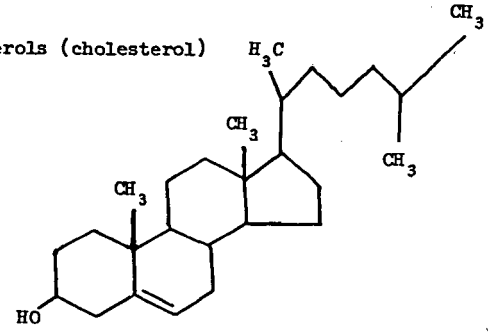
1. Triglycerides



2. Phospholipids (lecithin)



3. Sterols (cholesterol)



COMPONENT FATTY ACIDS:

A)	saturated	lauric	$CH_3(CH_2)_{10}COOH$
		Myristic	$CH_3(CH_2)_{12}COOH$
		palmitic	$CH_3(CH_2)_{14}COOH$
		stearic	$CH_3(CH_2)_{16}COOH$
		arachidic	$CH_3(CH_2)_{18}COOH$
B)	unsaturated	oleic	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$
		linoleic	$CH_3(CH_2)_4CH=CH-CH_2-CH=CH(CH_2)_7COOH$
		linolenic	$CH_3CH_2-CH=CH-CH_2-CH=CH-CH_2-CH=CH(CH_2)_7COOH$

TABLE 2

Food Composition (%) Cooked Edible Portions[8]

Component	Porterhouse steak (beef)	Lamb chop	Baked potato	Apple	Cod Fish	Cake Canned Salmon	Roast Chicken
Water	37.0	35.5	75.1	84.4	64.6	62.4	53.5
Protein	20.0	16.9	2.6	0.2	18.9	22.5	25.2
Ash	1.4	0.8	1.1	0.3	1.2	1.7	0.9
Total Carbohydrate	0.0	0.0	21.1	14.5	0.0	0.0	0.0
Fat	42.0	46.5	0.1	0.6	5.3	13.4	20.2
(% fat dry basis)	67.0	72.1	0.4	2.3	11.6	36.0	43.4

Lipid Composition (% of Total Fatty Acids)

Fatty Acids	Beef[9]	Salmon[10]	Potato[11]	Safflower oil[9]	Soybean oil[9]
Myristic	3.1	5.4	0.3	0.2	0.1
Palmitic	29.1	26.2	19.3	3.4	10.5
Stearic	18.9	6.3	5.4	5.3	3.2
Oleic	44.0	2.7	0.6	15.0	22.3
Linoleic	0.3	52.1	53.0	75.0	54.5
Linolenic	trace	1.8	19.7	0.4	8.3
Arachidic	trace	6.5	1.2	1.5	0.2
% of total lipid	98	10.0	11.5	95	98

Other than triglycerides or free fatty acids, foods also contain various amounts of other types of lipids as shown in Table 1. Phospholipids, the subject of much speculation as to their effect in oxidation, are basically composed of glycerol with two fatty acids and on the third position, a phosphoric acid connected to a nitrogen containing alcohol. They usually are associated with the mitochondria of a cell and are high in polyunsaturated fatty acids. Sphingolipids are similar to phospholipids but have sphingosine rather than glycerol as a base. There are many different sterols, such as cholesterol, but these are insignificant in terms of oxidation. Other lipid type materials include hydrocarbons, aldehydes, ketones, and tocopherols, the latter having an important role in the control of oxidation. In addition, many plants and some animal tissues contain carotenoid pigments which are either oxidized themselves or co-oxidized with lipids leading to unacceptability.

OVERALL PICTURE OF RANCIDITY

The real problem in discussing the rate or kinetics of lipid oxidation is defining what is meant by rancidity. One way to define rancidity is the development of an off flavor which makes the food unacceptable on a consumer market level. However, many different mechanisms produce various types of off flavors. What is of concern here are those flavors which are developed as a result of the reaction between oxygen and an unsaturated fatty acid, which may or may not be part of a triglyceride or phospholipid. The general picture of lipid oxidation with all the respective pathways is shown in Figure 1. The historical development of the free radical mechanism pictured here has been described in detail by Swern.[3] The major consequence is that once a free radical is produced (a compound having an unpaired electron), the high reactivity of the radical with oxygen causes a rapid conversion to a peroxide or hydroperoxide when considering food fats. These are quite unstable and can break down to produce more free radicals, thus initiating a chain reaction as in an explosion. It is this breakdown mechanism which is responsible for the rancid off flavors produced, as well as the myriad of other reactions which reduce shelf life as well as

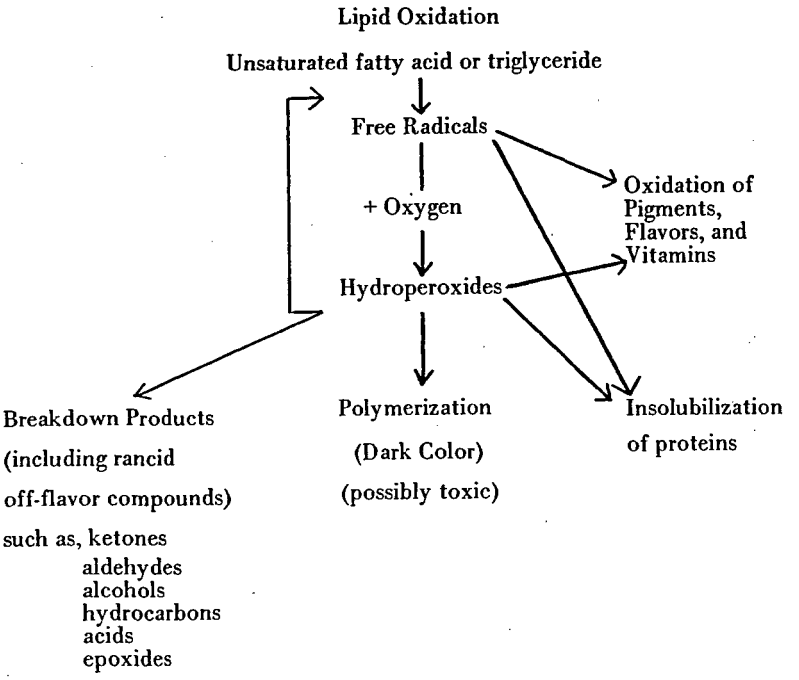


FIGURE 1. Overall mechanisms of lipid oxidation.

nutritional value. What makes this reaction so important, especially in its early stages, is that many of the small molecular weight compounds formed have very low odor threshold values. Thus, if they are objectionable in odor, only a few ppm or ppb are needed to give the food an unacceptable odor. Table 3 lists some threshold values of various compounds responsible for a rancid off odor. For example, hexanal has been implicated as a major breakdown off odor product in various foods, including dehydrated potatoes.[11,13] In oil, hexanal can be detected at 150 ppb (parts per billion) and in milk at 50 ppb. Considering this on a molar basis, this means that less than 0.00002% of the fat has to be oxidized in order to form an objectionable off odor. Thus, the kinetics of this early reaction are important unless other flavors and odors mask the rancid odor, or the odor is distilled off, such as in deep fat frying. The overall reaction is graphically displayed in Figure 2. Several interesting features of this will be examined later in detail in terms of the reaction kinetics. It should be noted that the most important feature is the noncorrespondence of peroxides and the early time in reaction extent when the product is considered rancid. This is due to the low flavor threshold values of the volatile aldehydes and ketones formed in the reaction.

Since these off flavor compounds can be detected in such early stages, it would seem that the other reactions shown in Figure 1 would not be important. However, the problem exists in that either the odor is masked or removed in some way, or that people have become accustomed to accept the rancid flavor. In many cases, foods are held under conditions where they become nutritionally inadequate or unsafe due to toxic compounds being formed. The area of nutritional value and toxicity has been reviewed extensively.[14-17] In addition, the free radical mechanism of lipid oxidation has been implicated in terms of fat

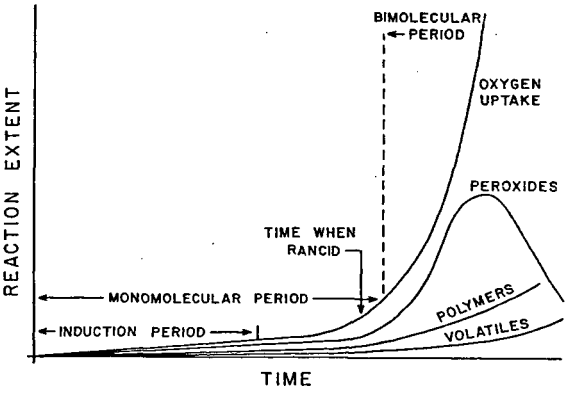


FIGURE 2. Extent of reaction as a function of time.

TABLE 3

Olfactory Perception Thresholds of Various Flavor Compounds Isolated From Dairy Foods (ppm)[310]

Compound	Flavor threshold value		
	Water	Milk	Oil
Acids			
Acetic	54		
Butyric	6.8	25.0	0.6
Hexanoic	5.4	14.0	2.5
Octanoic	5.8		350
Decanoic	3.5	7.0	200
Dodecanoic		8.5	700
Tetradecanoic			5000
Hexadecanoic			10000
Alcohols			
Butanol		0.500	
Hexanol		0.500	
Hexenol		0.01	
Octenol		0.01	
Maltol	7		
Furfurol		11.0	
Esters			
Methyl butanoate		0.500	
Ethyl butanoate		0.025	
Ethyl pentanoate		0.025	
Ethyl hexanoate		0.075	
Ethyl octanoate		0.5	
Ketones			
2-butanone		79.5	
2-pentanone		8.4	
2-hexanone		0.4	
2-heptanone		0.7	
2-octanone		0.5	
2-nonanone		3.5	
2-undecanone		15.5	
2-tridecanone		18.4	
Oct-1-ene-3-one		0.01	
Diacetyl			
Aldehydes			
Ethanal		1.2	
Propanal		0.43	1.6
Butanal		0.19	0.02
Pentanal		0.13	0.15
Hexanal		0.05	0.15
Heptanal		0.12	0.04
Octanal		0.07	0.46
Nonanal		0.22	0.32
Decanal		0.24	1.0
Undecanal			0.1
Dodecanal			0.46

Table 3 (continued)

Compound	Water	Milk	Oil
n-hex-c2-enal		0.07	
n-hex-t2-enal		0.10[s]	2.5
n-hex-c3-enal			0.1
n-hept-c4-enal			1.5×10^{-3}
n-hept-2-enal		0.077	0.63
n-non-2-enal		0.004	0.1
n-deca-2-enal		0.092	5.5
n-hepta-e,4-dienal		0.05	
n-nona-2,4-dienal			.01
n-deca-2,4-dienal	0.5×10^{-3}		0.28
methional		0.05	
5-hydroxy methylfurfural		1.0	
Lactones			
γ-decalactone		2	5
γ	.05	1.5	3

composition of the diet in relation to the process of aging. Pryor, among others, has worked in this field and extensively reviewed this area.[18-23] In July of 1971, a Gordon Research Conference discussed this area and its nutritional and biological implications. Although implications of the reaction are stunning, no one has direct evidence of proof yet. Roubal[181,182] claims a direct peroxyradical-protein interaction using electron paramagnetic resonance studies (EPR).

KINETICS OF THE FREE RADICAL MECHANISM

Although the mechanism of lipid oxidation has been reviewed elsewhere it is imperative from the standpoint of kinetics to reiterate the nature of the free radical mechanism. The complex reaction kinetics were postulated and studied in detail by Farmer et al.,[24] Bolland,[25] and Bateman.[26] It was found that the reaction was not just the simple addition of oxygen to a fatty acid to form an epoxide, as had been proposed earlier,[27] but a hydroperoxide was formed. Moreover, the reaction was involved between an unsaturated carbon-carbon double bond and oxygen, i.e., not a random addition on the chain of the lipid. In addition, several criteria of a free radical mechanism were met for the reaction:

1. The rate was not a direct function of the number of unsaturated groups (double bonds), but increased drastically as the number increased. Thus, linoleate oxidized 10 times faster than oleate, linolenate was 20 to 30 times faster.
2. The calculated quantum yield was greater than one.
3. Small amounts of various compounds either accelerated or inhibited the reaction in a drastic way.
4. Starting with pure material, a very long induction period occurred.
5. The reaction had a moderately high activation energy.

It should be noted that a free radical is any chemical species with an odd number of electrons, those of organic molecules being highly reactive.

Based on these criteria the reaction does not follow the simple rate law as in Equation 1,

$$-\frac{d(O_2)}{dt} = +\frac{d(ROOH)}{dt} = k\,(RH)\,(O_2) \qquad (1)$$

but rather the rate in Equation 2

$$-\frac{d(O_2)}{dt} = +\frac{d(ROOH)}{dt} = k\,(R_i)^{\frac{1}{2}}(RH)\,\frac{(O_2)}{(O_2) + k'(RH)} \qquad (2)$$

where

(RH)	=	substrate concentration
(O_2)	=	oxygen concentration
(ROOH)	=	peroxide concentration
k, k′	=	rate constants
t	=	time
$-\frac{d(O_2)}{dt}$	=	rate of oxygen uptake

$+\frac{d(ROOH)}{dt}$ = rate of peroxide production

R_i = rate of initiation

This had been deduced by assuming the reaction follows the proposed free radical pathway. This is described by dividing the reaction into three parts:

Initiation

$$\text{(Initiator)} \xrightarrow{k_i} \text{free radicals} \qquad \text{A.}$$

$$\text{Rate} = R_i$$

Propagation

$$R\cdot + O_2 \xrightarrow{k_o} ROO\cdot \qquad \text{B.}$$

$$ROO\cdot + RH \xrightarrow{k_p} ROOH + R\cdot \qquad \text{C.}$$

Termination

$$ROO\cdot + ROO\cdot \xrightarrow{k_t} \text{NON-RADICAL PRODUCTS} \qquad \text{D.}$$

$$ROO\cdot + R\cdot \xrightarrow{k_{t'}} \text{NON-RADICAL PRODUCTS} \qquad \text{E.}$$

$$R\cdot + R\cdot \xrightarrow{k_{t''}} \text{NON-RADICAL PRODUCTS} \qquad \text{F.}$$

$(R\cdot)$ = substrate free radical
$(ROO\cdot)$ = peroxyl free radical
k_i = initiation rate constant
k_o = oxygen step rate constant
k_p = propagation step rate constant
k_t, $k_{t'}$, $k_{t''}$ = termination rate constants

Obviously, this is a much more complex reaction than the direct addition of oxygen to the substrate. Details of the types and kinds of peroxides formed have been reviewed previously.[6] The reason for the increased rate with more highly unsaturated fatty acids is due to the increased sensitivity created by the methyl group between two double bonds. This activates the bond and upon attack a conjugated system is formed (i.e., double bond – single bond – double bond) which allows for resonance of the free radical and the myriad of products formed.

To solve for Equation 2 specifically, several cases are possible and several important assumptions (especially when we consider food stability later) are made. In the first place, it is assumed that the reaction can be treated by the Bodenstein Steady State Approximation which states that once the reaction begins, the concentration of each type of free radical is approximately constant, i.e., the rate of change is zero.

$$\frac{d(R\cdot)}{dt} = 0 \qquad (3)$$

$$\frac{d(ROO\cdot)}{dt} = 0 \qquad (4)$$

Under conditions where oxygen is not limiting one can assume initially that all the oxygen reacted is in the forms of peroxides. This implies that very little peroxide decomposition occurs practically since only a few ppm of the low molecular weight peroxide breakdown product is necessary for a food to be rancid. The solution based on the above becomes:

$$-\frac{d(O_2)}{dt} = +\frac{d(ROOH)}{dt} = \frac{k_p(R_i)^{\frac{1}{2}}}{(2k_t)^{\frac{1}{2}}}(RH) \qquad (5)$$

It is implicit in this assumption that Reaction B is so rapid that most or all of the free radicals are in the form of the peroxyl radical. Thus, most or all of termination takes place by peroxyl radical combination (Reaction D) and the other termination reactions have been assumed to be negligible.

When the partial pressure of oxygen is limiting, the rate equation solution is much more complex. To solve, it is usually assumed for mathematical sake that:

$$(k_{t'})^2 = k_t k_{t''} \qquad (6)$$

The solution then becomes:

$$-\frac{d(O_2)}{dt} = +\frac{d(ROOH)}{dt} = \frac{k_p(R_i)^{\frac{1}{2}}}{(2k_t)^{\frac{1}{2}}}(RH) \times \frac{(O_2)}{(O_2) + \frac{(k_{t''})^{\frac{1}{2}}}{(k_t)^{\frac{1}{2}}}\frac{k_p}{k_o}(RH)} \qquad (7)$$

It can be seen that if the second term in the

denominator is small compared to the oxygen concentration at high oxygen partial pressure then Equation 7 reduces to Equation 5 which is the case for no oxygen limitation. If the initiation rate is constant and the amount of substrate is high so that its concentration does not change substantially then Equation 7 can be manipulated as follows:

$$-d(O_2) = A\left[\frac{P_{O_2}}{P_{O_2} + B}\right] = R_o \qquad (8)$$

where

P_{O_2} = oxygen partial pressure
A, B = constants
R_o = rate of oxidation

then:

$$\frac{1}{R_o} = \frac{1}{A}\left[1 + \frac{B}{P_{O_2}}\right] \qquad (9)$$

Thus a plot of Equation 9 is a straight line as shown in Figure 3. As shown by Karel[28] in Figure 4, as the surface available to the oxygen atmosphere, divided by the total to volume of the oxidizable substate ratio increases, reducing the oxygen partial pressure has a decreasing effect on the decrease in the rate of oxidation. Thus, one would expect that for dehydrated foods, reduction in P_{O_2} may not be very effective, as will be seen subsequently. The temperature of the system is also very important in terms of the effect of oxygen partial pressure on rate. Figure 5 shows qualitatively that as temperature increases, the rate becomes more limited with respect to oxygen, especially for linoleate.[26,32]

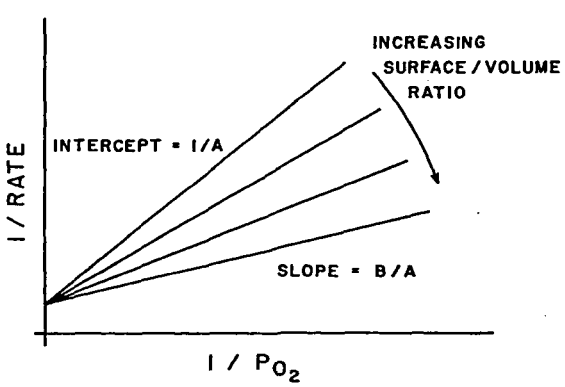


FIGURE 4. Effect of surface area and oxygen pressure on the relative rate of oxidation.

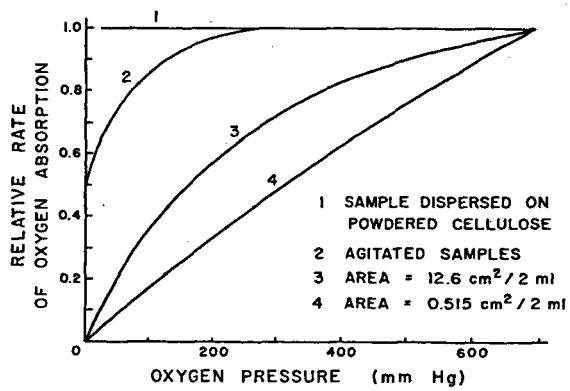


FIGURE 3. Graphical representation of the effect of oxygen partial pressure on rate of oxidation.

This is due to the reduced solubility of oxygen as temperature increases. Thus, accelerated storage tests (i.e., high temperature) become of limited value if done at low oxygen pressure. Reaction pathways also change with oxygen partial pressure in terms of termination reactions as seen qualitatively in Figure 6. Measurements of certain end products may have little meaning if studies are carried out at different oxygen levels. Marcuse[32] has done extensive work on oxygen level effects on kinetics of linoleate oxidation and has confirmed all the above kinetic predictions.

One sees, therefore, that the normal lipid oxidation pathway is very complex and is affected by temperature, oxygen, and type of substrate. How this can be utilized to analyze actual oxidative deterioration in foods will be discussed below.

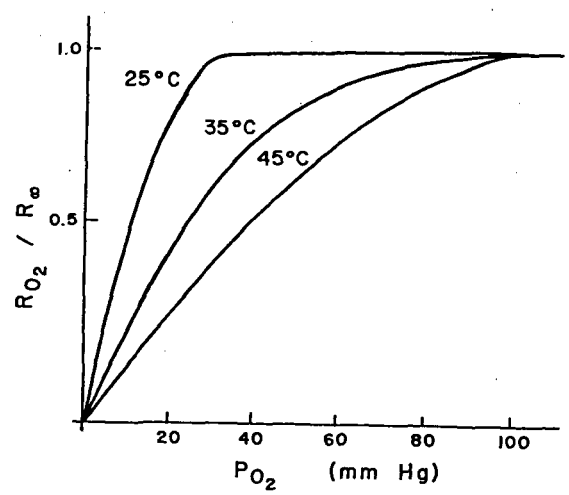


FIGURE 5. Effect of temperature and oxygen pressure on the relative rate of oxidation.

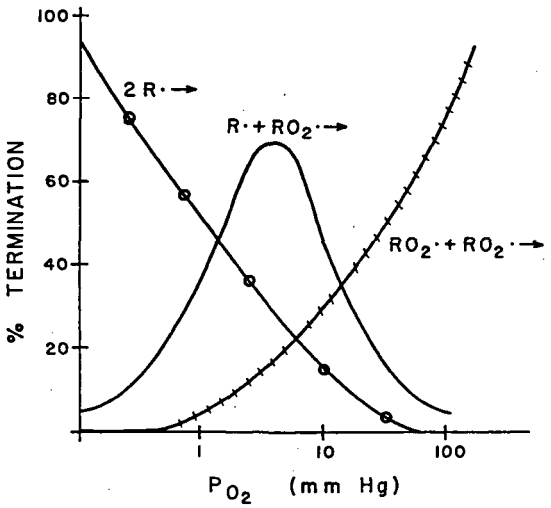


FIGURE 6. Effect of oxygen pressure on the type of termination occurring in the free radical oxidation reaction.

TYPICAL BREAKDOWN PATHWAYS

From the standpoint of food oxidation, the important lipids are the unsaturated fatty acid moieties, particularly oleate, linoleate, and linolenate, the predominant ones in foods. Pathways for their oxidation have been summarized in detail elsewhere.[6] Figure 7 shows the typical pathway for linoleate which has three major resonance forms of the peroxyl radical and so three major peroxides are formed. Due to the stabilization of the radical in a conjugated system, the C_9 and C_{13} peroxides account for most of the peroxides (95 to 98%).[29] The interaction and breakdown of these peroxides by various mechanisms account for the myriad of compounds formed which can contribute to the off odor of foods.

The major types of reactions occurring in peroxide breakdown can be classified as follows:[30]

1. Cleavage

$$R_1\text{-}\underset{\underset{OOH}{|}}{CH}\text{-}R_2H \xrightarrow{fast} R\text{-}\underset{\underset{O\cdot}{|}}{CH}\text{-}R_2 + \cdot OH \qquad G.$$

This is a very fast step producing two highly reactive radicals. The fatty acid oxy-radical is especially reactive and predominantly goes through a cleavage followed by reaction in the propagation step.

2. Propagation (see Equation H. below)

In each case an additional R· radical is also produced, carrying on the propagation reaction. If the cleavage radical products enter the normal propagation step (since the substrate RH is in excess), various types of compounds are formed. Hexanal and pentane can be formed from linoleate which are volatile and can be detected organoleptically at low concentrations (Figure 7).

If the radical reacts directly with the substrate, an unsaturated alcohol can form:

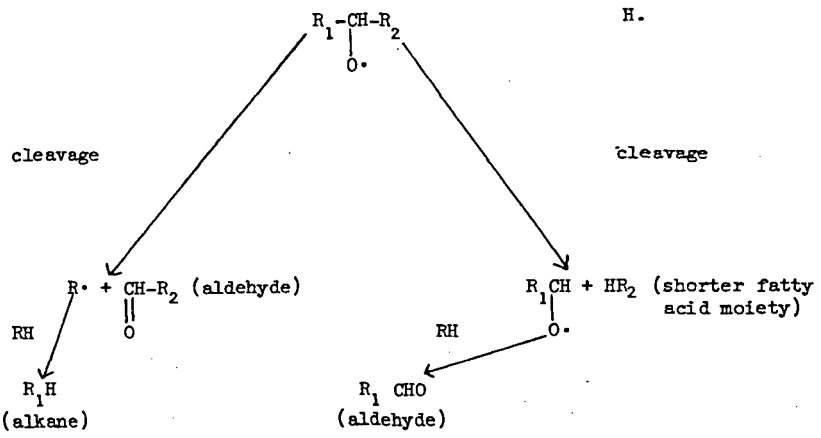
$$R_1\text{-}\underset{\underset{O\cdot}{|}}{CH}\text{-}R_2 + RH \dashrightarrow R_1\text{-}\underset{\underset{OH}{|}}{CH}\text{-}R_2 + R\cdot \qquad I.$$

This would occur again in the normal propagation step.

3. Termination

Several termination reactions are also possible, producing ketones or alcohols:

$$R_1\text{-}\underset{\underset{O\cdot}{|}}{CH}\text{-}R_2 + R\cdot \longrightarrow R_1\text{-}\underset{\underset{O}{||}}{C}\text{-}R_2 + RH \qquad J.$$



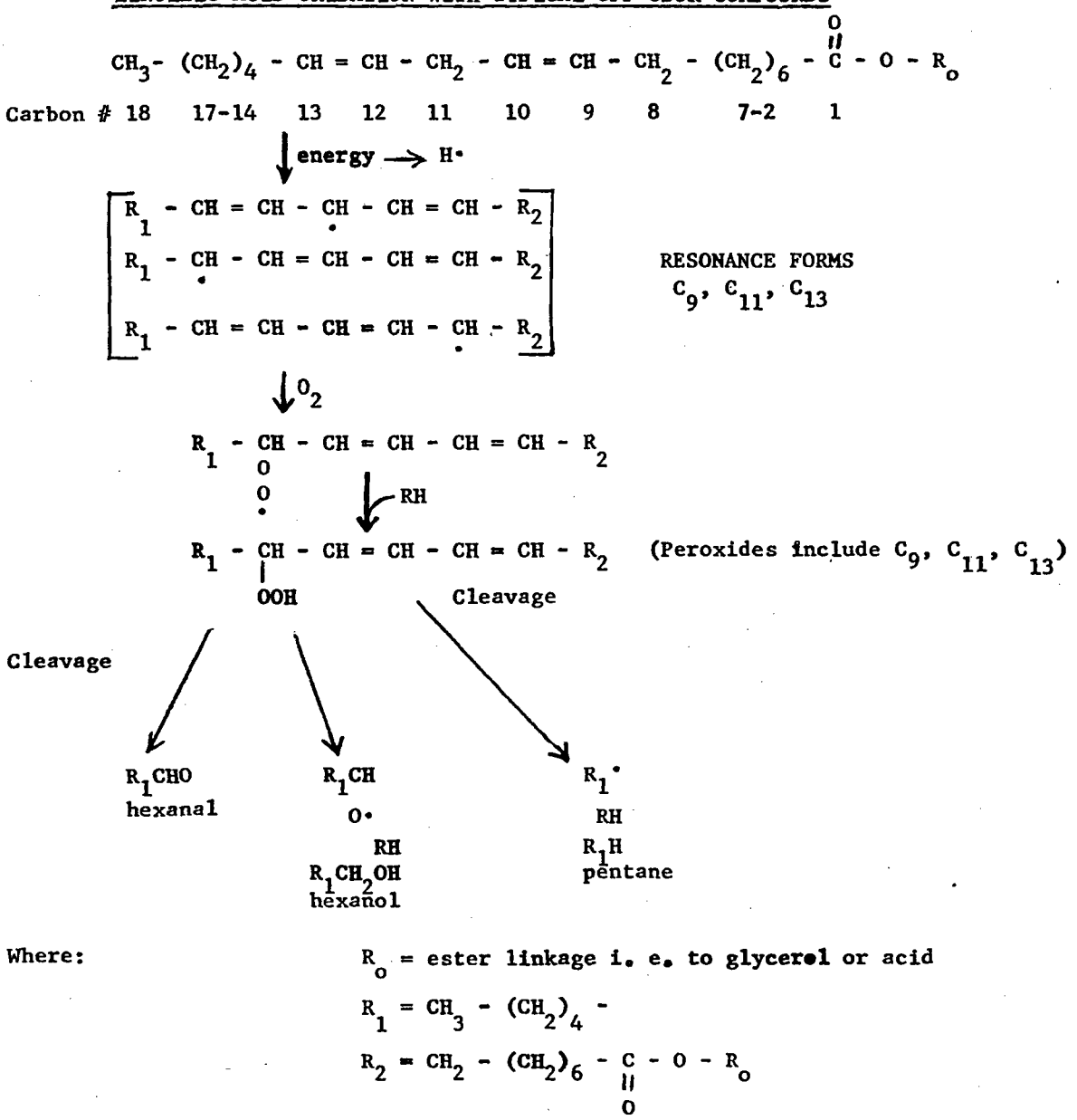


FIGURE 7. Typical breakdown pathway for oxidation of linoleic acid.

$$R_1\text{-}\underset{\underset{O\bullet}{|}}{CH}\text{-}R_2 + RO\bullet \longrightarrow R_1\text{-}\underset{\underset{O}{||}}{C}\text{-}R_2 + ROH \text{ (alcohol)} \quad K.$$

or higher molecular weight polymers:

$$R_1\bullet + R_2\bullet \longrightarrow R_1\text{-}R_2 \text{ (polymer)} \quad L.$$

$$R_1O\bullet + R_2\bullet \longrightarrow R_1OR_2 \text{ (ether)} \quad M.$$

$$R_1O\bullet + R_2O\bullet \longrightarrow R\text{-}O\text{-}O\text{-}R_2 \text{ (peroxide)} \quad N.$$

In addition, epoxide formation is possible through the following sequences:[27]

$$ROOH + R_1\text{-}CH = CH\text{-}R_2 \longrightarrow R_1\text{-}\underset{\underset{O}{\diagdown\diagup}}{CH}\text{-}CH\text{-}R_2 + ROH \quad O.$$

$$ROO\cdot + R_1\text{-}CH = CH\text{-}R_2 \longrightarrow R_1\text{-}\underset{O}{CH\text{-}CH}\text{-}R_2 + RO\cdot \quad P.$$

Table 4 lists some typical breakdown products found in rancid foods and the hydroperoxide from which they are derived. This review will not deal specifically with this area, as it is quite vast owing to the many mechanisms possible and those that have been proposed. Since linoleate is the predominant polyunsaturated fatty acid in most foods and oxidizes 10 to 15 times faster than oleate, hexanal is useful as a measure of the degree of oxidation.[11,31]

From a storage standpoint, the breakdown of saturated fatty acids through oxidation can be neglected. Thaler et al.[33-35] have studied the autoxidation of lauric and stearic acid. To get measurable peroxides and secondary products, the experiments had to be carried out at 130 to 150°C. It was interesting to note that the methyl esters gave higher peroxide values, indicating that the acids catalyzed peroxide decomposition. The degradation products were saturated alkan-2-ones of one carbon less than the initial lipid, indicating that primarily β oxidation takes place. Some alkanals also were present, but in trace amounts. Thus, a random attack does not occur on the chain, but is confined to the second carbon from the ester or acid linkage. Brodnitz[36] recently has reviewed the field of oxidation of saturated fats. It has been found that at 100°C the rate of saturated fat oxidation is about 100 times slower than for unsaturated linoleate and is essentially unmeasurable under normal storage conditions. Therefore, even though flesh foods may contain 40% of the fat as saturated, these can be essentially treated as nonreactive material below 50 to 60°C. For example, at 60°C methyl palmitate showed no peroxides after holding for 2 months in air.[37] However, in the presence of oxidizable or oxidized fat, the free radicals generated could react with the saturated fats.[37] A multitude of saturated products could be formed at normal storage temperatures, as in milk and corn lipid oxidation.[38-41] This is also supported by the fact that direct rupture of a C-H bond requires about 35 to 40 kcal/mole for direct attack by oxygen, whereas it is much less for a peroxyl radical attack.[4]

TABLE 4

Specific Breakdown Products

Fatty Acid	Hydroperoxide	Aldehyde Formed
Oleate	C_8	un-dec-2-enal
	9	2-decenal
	10	nonanal
	11	ocatanal
Linoleate	C_9,	2, 4-decadienal
		2-octenal
	C_{13}	n-hexanal
Linolenate	C_9	2, 4, 7-decatrienal
	12	2, 4-heptadienal
	13	3-hexenal
	16	propanal

FACTORS INFLUENCING INITIATION OF RANCIDITY

Primary Initiation

In terms of the onset of rancidity in food lipids the most important problem is that of initiation. Initiation brings up the proverbial question of "what came first, the chicken or the egg?" This implies that since direct attack of oxygen on an unsaturated fatty acid seems to be improbable thermodynamically, something must occur to help the formation of the first few peroxides.[4] Once these are formed, the chain can begin to propagate. A major question to be asked is how these first peroxides are formed.

As was implied above in the reaction:

$$RH + O_2 \longrightarrow \text{free radicals or ROOH} \quad Q.$$

has a very high activation energy (~ 35 to 65 kcal/mol),[45] so that if a peroxide is to form, something must assist it. Recent work[42,43] has been centered around the possible role of singlet oxygen in the primary initiation reaction, especially in studies of ultrapure lipid material containing no measurable peroxides.[45] Hydroperoxide formation requires a change in total electron spin since both the substrate and product are in singlet states, while oxygen is a triplet. Conservation of spin is violated, making the reaction improbable.[46] Rawls et al.[43,44] postulated if singlet oxygen was the species involved, then the reaction is possible for formation of original hydroperoxides. Singlet O_2 can be formed through photo-chemical reactions in the presence of a sensitizer:

$$S + h\nu \longrightarrow {}^1S^* \longrightarrow {}^3S^* \quad R.$$

$^3S^* + {}^3O_2 \longrightarrow {}^1O_2^* + {}^1S$ S.

$^1O_2^* + RH \longrightarrow ROOH$ T.

$ROOH \longrightarrow$ free radicals

where

^1S = singlet state sensitizer
$^1S^*$ = excited singlet state sensitizer
$^3S^*$ = excited triplet state sensitizer
$^3O_2$ = normal triplet oxygens
$^1O_2^*$ = excited singlet state oxygen
$h\nu$ = UV light energy in photons

It was found that singlet oxygen reacted about 10^3 to 10^4 times faster than normal oxygen with methyl linoleate. The normal plant and tissue pigments such as chlorophyll, pheophytin, and myoglobin can act as the sensitizers. The peroxide formed was nonconjugated, so the normal UV technique used to measure oxidation at low values would not be adequate. These nonconjugated products are produced in small quantities and once oxidation starts they disappear rapidly, so only conjugated fatty acids are found, even when the oxygen reacted is unmeasurable.[45]

Uri[47,48] and Heaton and Uri[49] have postulated that trace metals are responsible for primary initiation and analyzed the problem thermodynamically. Metals that are oxidized by one electron transfer are the most active. This fits in very well with the modern singlet state oxygen theory, since most of the sensitizers are bound metal complexes which can undergo this oxidation and may be more active than free metals due to geometrical considerations.

In addition, it has been proposed that increased temperature can be responsible for direct attack of oxygen.[50-52] Perhaps at these higher temperatures the singlet-state O_2 formation increases. Of course, ultraviolet light would be a good initiator under this hypothesis, if the proper sensitizers were present. Chahine and deMan find very rapid oxidation of corn oil in UV light.[53] A calculation of the activation energy from their results shows a value of about 4 kcal/mole, greatly reduced from the 35 to 65 kcal/mole predicted for RH, O_2 interaction. Again this supports singlet oxygen initiation, possibly through the photosensitization with the trace chlorophyll in the corn oil or the free metals.

Heaton and Uri[49] have some evidence for a direct metal-substrate reaction:

$RH + M^{+n} \longrightarrow R\cdot + H^+ + M^{+(n-1)}$

Kamiya et al.[54] believe that the data are insufficient to support this since it would assume that the rate is a function of the substrate concentration. The evidence for this is lacking. However, Bawn[55] has shown this to be a possibility for short chain aldehydes and ketones which complex with the metal. As carbon chain length increases, especially below 60°C, this can be eliminated; this reaction unimportant in food storage. The results of Heaton and Uri[49] can be explained by the singlet state oxidation theory if it is assumed the metal stearate added acts as a sensitizer.

Dulog[56,57] has studied the primary rate of autoxidation with tetralin and other unsaturated compounds. He used a very special polarographic method to estimate the initial rate. By back extrapolation he found the initial rate to be at least 10^5 to 10^6 times slower than for monomolecular decomposition at 25°C and about 10^3 times slower at 70°C. If the presence of singlet state oxygen for initiation is assumed, its concentration is very small under conditions protected from UV light, or in a food tissue. This leads to very long induction times found for many foods before oxidation proceeds at a measurable rate. Conversely, if the foods are held under improper conditions so that some initiation occurs (or there is a high metal content free or bound) at the start of storage, enough peroxides are present so that shelf life is limited.

Monomolecular Decomposition

It can be assumed that once the first hydroperoxides are produced, the chain reaction takes over (e.g., Bolland and Gee[58] find 100 peroxides formed for each initiation: chain length = 100). By the time many foods go into storage, sufficient peroxides are present so that initiation proceeds by a process which requires less energy; the monomolecular decomposition into free radicals.

$ROOH \longrightarrow RO\cdot + \cdot OH$ U.

or

$ROOH \longrightarrow R\cdot + HO_2\cdot$ V.

The reaction is usually written as if it were metal catalyzed. Thus

$$ROOH + M^{+n} \longrightarrow ROO\cdot + H^{+} + M^{+(n-1)} \quad W.$$

or

$$ROOH + M^{+(n-1)} \longrightarrow RO\cdot + OH^{-} + M^{+n} \quad X.$$

It can be seen that by the latter scheme the metal in the oxidized state can always be regenerated and show true catalysis. Uri[4] believes that removal of trace metal impurities is impossible from a lipid, so metal catalysis always occurs. This is also supported by the fact that the initiation activation energy calculated for a pure lipid system is about 30 to 40 kcal/mole, whereas measured activation energies, especially when no amounts of metals are present, are about 20 kcal/mole.[25,26]

Based on a metal catalyzed decomposition of hydroperoxide and substituting into Equation 5, Maloney et al.[59] and Labuza et al.[60] have shown that Equation 5 can be simply reduced to:

$$-\frac{d(O_2)}{dt} = \frac{d(ROOH)}{dt} = \frac{k_p k_i^{\frac{1}{2}}}{(2k_t)^{\frac{1}{2}}} (M)^{\frac{1}{2}} (ROOH)^{\frac{1}{2}} (RH) \quad (10)$$

where k_i = monomolecular initiation rate constant and (M) = catalyst concentration. Since this reaction occurs at low extents of oxidation, (RH) can be assumed constant. If the metal acts as a true catalyst, its concentration also does not change. Thus, these all can be lumped into a single constant K_m for the monomolecular periods where:

$$K_m = \frac{k_p k_i^{\frac{1}{2}}}{(2k_t)} (M)^{\frac{1}{2}} (RH) \quad (11)$$

Therefore, the rate of oxidation or peroxide production is:

$$-\frac{d(O_2)}{dt} = +\frac{d(ROOH)}{dt} = K_m (ROOH)^{\frac{1}{2}} \quad (12)$$

A plot of the rate of oxidation vs. the square root of the extent should give a straight line up to the point where either the substrate concentration decreases significantly or the peroxides significantly decompose into secondary products. These kinetics should apply up to an oxidation extent of at least 0.01 to 0.02 mole/mole.[26,61-65]

Since it is impossible to determine the individual rate constants of Equation 10 unless one uses the special rotating sector techniques which are not applicable to nonliquid foods, it is much easier to use the integrated form of Equation 12 to get an overall rate constant. Maloney et al.[59] and Kern and Dulog[64,65] showed the integrated form of Equation 12:

$$\int_0^{[ROOH]} \frac{d(ROOH)}{(ROOH)^{\frac{1}{2}}} = \int_0^t K_m \, dt \quad (13)$$

$$(ROOH)^{\frac{1}{2}} = \frac{K_m}{2} t \quad (14)$$

Equation 14 is easier to use and does not require taking slopes of the extent vs. time curve to get rates. Therefore, on a plot of the square root of the extent vs. time, the slope of the line is equal to $K_m/2$ and thus K_m can be found directly. Before looking at the magnitudes of K_m in lipid and food oxidation, the bimolecular period and the effects of metals will be discussed. For reference in pure lipids, K_m is on the order of 10^{-2} to 10^{-3} $(moles/mole)^{\frac{1}{2}}$ hr^{-1} at 30 to 40°C.

Bimolecular Period

After a period of time during the autoxidation of an olefin or unsaturated lipid, the concentration of hydroperoxide builds up to a point at which a change in the initiation mechanism occurs. As stated above, monomolecular decomposition of single hydroperoxides occurs up to 0.5 to 1% oxidized on α molar basis. In this period the rate and time course is proportional to the square root of the extent of oxidation. Most likely, foods would become unacceptable in this rate period because of the very low thresholds observed for off flavor compounds (Table 3). However, this has not been confirmed quantitatively.

Beyond this, it is found that the rate is directly proportional to the extent of peroxide concentration which leads to a proposed bimolecular initiation reaction:

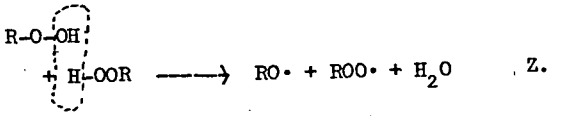
$$2\ ROOH \longrightarrow ROO\cdot + RO\cdot + H_2O \quad Y.$$

If this reaction is catalyzed by trace metals, it is similar to the combination of the two monomolecular initiation reactions with the metal acting as a true catalyst.

Modern spectroscopic techniques have shown that there is more to the reaction than just a two-step process. Hiatt et al.[66] observed a hydrogen bonded complex of two peroxides for

oxidation of t-butyl hydroperoxide and tetralin hydroperoxide. Heaton and Uri[49] observed association for metal catalyzed linoleic acid, as did Shelton and Vincent.[52] Bateman[26] also found this by using IR techniques, as did Walling and Heaton.[67]

As discussed by Uri[4] and originally proposed by Bateman,[26] once the peroxide composition is high enough, the peroxides are in close enough vicinity to be able to associate. Diffusion limitations are overcome and the observed kinetics would follow. Semenov[68] analyzed the reaction thermodynamically and observed that a hydrogen bonded dimer decomposition was energetically favorable to give the observed kinetics. This is illustrated below



According to his calculations it is interesting to note that the reaction should proceed slower as the polarity of the solvent increased. This would be due to hydrogen bonding of the peroxide to the solvent and has interesting implications in terms of actual foods as will be seen.

In order to get a measure of the magnitude of the overall rate during bimolecular decomposition a similar treatment of Equation 5 is made substituting Equation 15 for the initiation rate.

$$R_{ii} = k_{ii}(ROOH)^2 (M) \qquad (15)$$

where k_{ii} = bimolecular initiation rate constant. Thus for non-oxygen limiting situations:

$$-\frac{d(O_2)}{dt} = +\frac{d(ROOH)}{dt} = \frac{k_p k_{ii}^{1/2} (ROOH)(M)^{1/2}}{(2k_t)^{1/2}} (RH) \qquad (16)$$

Under situations where peroxide decomposition is still not significant Equation 16 can be integrated as follows:

$$\text{set } y = (ROOH) \cong (O_2) \qquad (17)$$

$$(RH) = (RH)_o (1 - y) \qquad (18)$$

where (RH_o) = initial substrate content

$$K_B = \frac{k_p k_{ii}^{1/2} (RH)_o}{(2k_t)^{1/2}} = \text{bimolecular decomposition rate constant} \qquad (19)$$

$$\therefore \frac{dy}{dt} = K_B y (1 - y) \qquad (20)$$

$$\log_e \frac{y}{1 - y} = K_B t \qquad (21)$$

A plot of the $\log_e$ of the corrected oxygen absorbed (i.e., the amount substrate reacted divided by the unreacted substrate) vs. time gives a straight line. The straight line relationship is shown in Figure 8 for the oxidation of methyl linoleate at 37°C.[69] It is interesting to note that in some cases above 5 to 10% oxidized, the bimolecular period does not hold either. This occurs when peroxide breakdown becomes significant, but should take place long after organoleptic unacceptability. For methyl linoleate (Figure 8) the measured K_B at 37°C is about 10^{-2} hr^{-1}.

Individual Rate Constants

As noted previously, it is impossible to measure the individual rate constants in foods; however, in pure oils and lipids this is possible. Uri[4] has discussed the details of the required techniques. Howard and Ingold[70] and Kamiya et al.[71-73] have shown the application of the method to many olefin oxidations. Basically, the method consists of measuring a light and dark reaction of the olefin with oxygen in an apparatus in which a rotating disk regulates the application of light on the reaction vessel. Usually, some known initiator, such as benzoyl peroxide, is used. Based on the above studies, it is important to note that:

1. k_p the propagation rate constant is a strong function of the substrate and has an activation energy (E_a) of about 3 to 5 kcal/mole. It is not affected very much by the solvent system (Table 5).

TABLE 5

Effect of Solvent on Oxidation Constants (tetralin oxidation)[71]

Solvent	Dielectric constant	k_p	$k_t \times 10^{-5}$
n-decane	2.0	4.6	40
Chlorobenzene	5.6	6.4	38
Acetonitrile	39	8.0	16
Dimethylsulfoxide	45	6.6	7.6

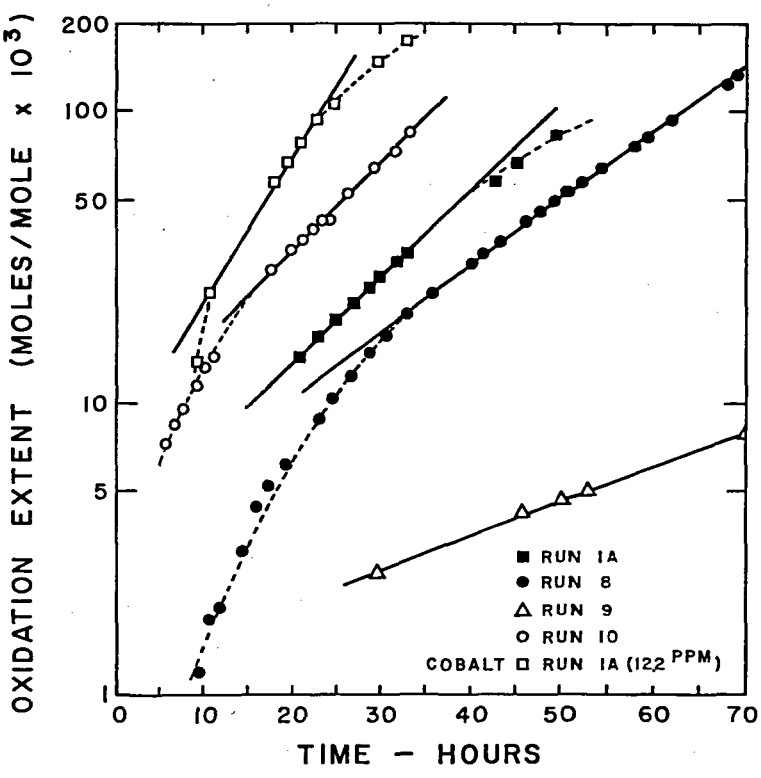


FIGURE 8. Bimolecular rate period plot of log (extent) vs. time.

2. k_o is very large for nonoxygen limiting kinetics and has an E_a close to zero.

3. k_{ii} is about 10 times larger than k_i and has an activation energy of 25 kcal/mole as compared to 30 kcal/mole for linoleate. These large activation energies make initiation the rate controlling step.

4. k_t, k_t', k_t'', do not change much with the substrate. The surrounding solvent system, however, does affect k_t moderately.[70] This can be seen in Table 5. All termination reactions have an activation energy of from 5 to 8 kcal/mole. This may be of consequence in product formation.

5. The assumption that $(k_t')^2 = k_t\, k_t''$ is not very good for study of low oxygen concentration being off by a factor of three to five for some olefins.[70-72]

Table 6 lists some overall rate constants K_m and K_m calculated from combining either the individual constants or by applying the above equations. Their relative differences are slight considering the variety of methods used and the types of systems tested.

MAXIMUM RATE KINETICS

One consideration that should be given to foods is the phenomenon of maximum rate kinetics as proposed by Mesrobian and Tobolosky.[74] In many cases food oxidizes at a fairly constant rate and the measured peroxides fall far below the measured oxygen reacted. This is in contrast with the work of Lundberg and Chipault[75] where for pure methyl linoleate the PV (peroxide value in concentration terms) closely follows oxygen uptake and also the work of Martinez et al.[10]

Under maximum rate kinetics (this does not imply that oxidation is going on at a maximum rate, but rather the peroxide content has reached a relatively constant value) one cannot assume that oxygen absorption is equal to peroxide production. Rather, for peroxides it is found that

$$+\frac{d(ROOH)}{dt} = 0 \qquad (22)$$

For the monomolecular period, assuming significant peroxide decomposition:

TABLE 6

Overall Oxidation Constants

System	T°C	K_M moles/mole$^{1/2}$hr^{-1}	K_B hr^{-1}	Ref.
Bulk Methyl Linoleate	30°C		2.5×10^{-2}	64, 65
	40°	13×10^{-3}	6.4×10^{-2}	64, 65
	50°C	40×10^{-3}	16.6×10^{-2}	64, 65
Bulk methyl linoleate	37°C	10.9×10^{-3}	6.6×10^{-2}	60
[Maximum rate – 200-500 ul/gram hour]				
Bulk ethyl linoleate	40°C	3×10^{-3}	3×10^{-2}	26
Methyl linoleate in Emulsion	40°	32.3×10^{-3}	8.5×10^{-2}	185
	45°	56.1×10^{-3}	13.2×10^{-2}	
	50°	95.5×10^{-3}	20.7×10^{-2}	
Methyl linoleate/Cellulose Powder	37°C			60
	Dry	7.1×10^{-3}	8.2×10^{-2}	
	32%RH*	5.4×10^{-3}	5.3×10^{-2}	
	50%RH	4.6×10^{-3}	5.0×10^{-2}	
[Maximum rate – 450 ul/gram hour]				
Tri-linoleate/cellulose powder	55°C			60
	Dry	1.74×10^{-3}	0.82×10^{-2}	
	32%RH	1.25×10^{-3}	0.74×10^{-2}	
	50%RH	1.12×10^{-3}	0.58×10^{-2}	
Methyl linoleate/Filter paper	37°C			60
	Dry	7.0×10^{-3}	4.6×10^{-2}	
	98%RH	20×10^{-3}	6.8×10^{-2}	

*Relative humidity

$$+\frac{d(ROOH)}{dt} = \frac{k_p k_i^{1/2}}{(2k)^{1/2}} (M)^{1/2} (ROOH)^{1/2} (RH) - k_i (M) (ROOH) \quad (23)$$

Combining Equations 22 and 23, for peroxide value we get:

$$(ROOH)_{max} = \frac{k_p^2 (RH)^2}{2k_t k_i M} \quad (24)$$

or the rate becomes:

$$-\left[\frac{d(O_2)}{dt}\right]_{max} = \frac{k_p^2 (RH)^2}{2k_t} \quad (25)$$

It can be seen that the rate is dependent only on substrate concentration which falls slightly with time. The same Equation 25 is found when bimolecular peroxide decomposition is assumed. However, $(ROOH)_{max}$ is found to be the square root of Equation 24 i.e., a lower value. In either case, trace metal concentration does not affect the oxidation rate. In no case have these kinetics been applied to foods, but it would seem to be applicable in light of the kinds of data found. In addition, these kinetics may explain the very rapid constant rates found in hematin catalyzed oxidations where no induction period occurs.[76, 77]

COMPETITIVE OXIDATION

One last complicating factor that occurs in pure systems and thus makes analysis of lipid oxidation of foods almost impossible is competitive oxidation. The initial work by Russel[78] showed that in mixing a fast oxidizing substrate with a slow one, the expected dilution effect did not occur. In fact

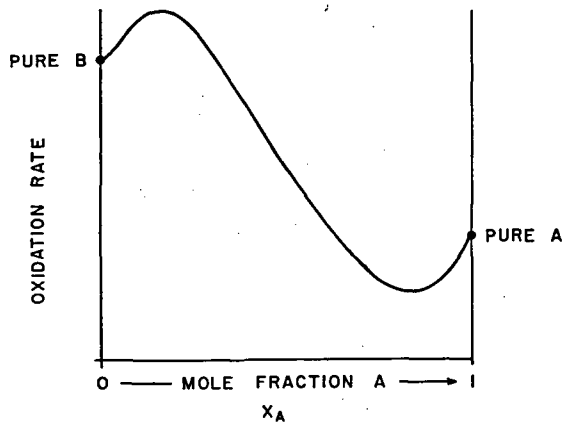


FIGURE 9. Effect of addition of a competitive oxidation species on overall rate of oxidation.

both pro-oxidant and anti-oxidant phenomena were observed as seen diagrammatically in Figure 9. Thus, the addition of a small amount of highly reactive substrate slows the oxidation rate and vice-versa, due to cross termination steps. This has been verified for many substrates by various workers.[79-84] Mayo[85] has recently reviewed this area and explained the cross termination reactions and the fact that the overall rate constant (K_m of the above derivation) was a better index to use in the analysis of the mechanisms of competitive oxidation. The major effect was attributed to the reactivity of the different peroxy radicals in termination steps.

No recent work has extended this to the types of lipids in food. Old data[86] showed that oleate acts merely as a diluent for linoleate oxidation. However, the concentrations studied and test procedures used may have missed the accelerating effect of oleate. If this does occur, then analysis of lipid oxidation kinetics in foods is quite complicated since:

1. Foods do not contain just one type of unsaturated fatty acid
2. Some foods such as fish contain small traces of very highly oxidizable fats.

Buttery et al. for example, finds that linoleate disappears twice as fast as linoleate in dry potato gomules. Unless one makes actual measurements of the fatty acids with time, then from a kinetic standpoint, the application of the overall rate constant would be best for forecasting of shelf life.

METAL CATALYSIS AND LIPID OXIDATION

General Catalytic Effects

Ingold[7] thoroughly reviewed the area of the kinetics of metal catalyzed oxidation in 1962. The transition (heavy) metals which possess two or more valency states with a suitable oxidation-reduction potential between them, both decrease the induction period and increase the rate of oxidation. These metals include cobalt, iron, copper, nickel, and manganese, as well as others of minor importance. The major action is in the reduction of the initiation step activation energy down to 15 to 25 kcal/mole. The mechanism of interaction of the metals with peroxides has been of major interest to many theories. Ingold[7] feels that the reduction step, M^{+n} going to $M^{+(n-1)}$ is the slow step which is followed very rapidly by the oxidative step. However, regeneration is not complete because of other reactions such as complexing with chain termination products or water if present.

Ochiai[87] studied magnesium catalyzed oxidation of cyclohexene. Using IR, ESR, and visible spectroscopic data it was concluded that the hydroperoxide entered the coordination sphere of the metal. The proposed reaction was of a Haber-Weiss mechanism through decomposition of an intermediate product as follows:

$$ROOH + M^{+n} \rightleftharpoons (ROOH - M^{+n}) \longrightarrow AA.$$
$$ROO\cdot + M^{+(n-1)} + H^{+}$$

In his studies with metallophthalocyanine compounds, those with a porphyrin ring type structure, he concluded that several other metal reactions were possible. These include catalyst decomposition, activation of molecular oxygen possibly to singlet oxygen, and direct radical initiation with substrate. This was based on the electronic structure of the coordination shells of the metals. In a porphyrin structure a change in valence of the metal is influenced by the whole coordination structure. Possami et al.[88] recently reported on the hemoglobin-oxygen reaction in which singlet state oxygen was proposed to be produced. This oxygen then could cause the oxidation of a lipid substrate. Waters[89] also supports the singlet state

reaction caused by metals. He feels that the reduction of the metal is not favorable since they usually exist in the higher oxidized form in solution. He also feels that in systems containing water, the peroxide may displace the water from the coordination shell of the metal and enter the ligand shell, as Ochaia found. Then the peroxide in the shell is in close coordination with the metal so that cleavage reactions become favorable to form free radicals. This has important bearings on rancidity development in meats, as will be discussed.

In most test tube studies of oxidation of pure lipids, the added catalyst concentration is usually high (200 to 2000 ppm).[71-73,90] In these cases, the normal induction period is not observed and the rate attains a constant value, then slowly decreases. This suggests that maximum rate kinetics should be applied, since the peroxide value for this to occur decreases as catalyst concentration increases (Equation 24). There no longer is the expected 1:1 ratio of peroxide to oxygen reacted even at the start of the reaction and application of normal kinetics, as many researchers have done in error.[49] Chalk and Smith[91] correctly analyzed autoxidation of cyclohexene as maximum rate kinetics.

Tappel[92-94] investigated the role of heme catalyzed oxidation of lipids. With linoleic acid in emulsion, it can be found from analyzing his data that maximum rate kinetics could be applied. Brown et al.[97] got similar results with heme catalyzed menhaden oil oxidation. An interesting phenomenon is that at very high catalyst concentrations an inhibition of the rate might occur. Lewis and Wills[96] reported this for heme and concluded that a stable metal-hydroperoxide complex formed. Chalk and Smith[98] found this for a series of various chelated metal complexes in autoxidation of several olefins. Betts and Uri[99] proposed that this reaction was the formation of an inactive bidentate chelate of the type $(MX_2)_2 \cdot 2RO_2$ in which the ROO· free radicals act as bridges.

It can be seen that state of the trace metal catalysis is quite important in foods, since they seriously alter the rate and course of the reaction. For example Watts[95,100] found that heating meat to 110°C destroys the heme structure which cause a faster development of rancidity. Heating vegetable oils releases catalytically active metals from inactive bound complexes to also accelerate lipid oxidation.[101] Chalk and Smith[98] have shown that bound metal complexes can either have no effect, be catalytic in nature, or behave as reaction inhibitors. Allan and Wood[102] have found that in linoleic acid emulsions, oxidation rates are accelerated with added ascorbic acid at low copper concentrations, but this decreased as ascorbic acid was increased. Similar results were found by Hasse and Dunkley.[103,104] In contrast to this Marcuse,[105] Smith and Dunkley,[106] and King[107] report that ascorbate acts as an antioxidant, thus confirming the fact that some knowledge of the state of the metal and its coordination sphere must be known if the action of an additive is to be understood. Similar contradictory results are found in studies with histidine. For example, Karel et al.[108] finds histidine to be an antioxidant for linoleate whereas Saunders et al.[109] find a pro-oxidant effect. The major difference is that the former study was done in dry systems, while the latter in an aqueous emulsion. This suggests that the state of the solvent system around the lipid and metal is also important.

Coordination Sphere, Solvent System and Catalysis

Modern quantum mechanics and electronic theory have never been applied to the study of lipid oxidation of foods, but as suggested by Waters,[89] it is a necessity for understanding the reaction. Based on published work, several conclusions can be drawn which will help to understand lipid oxidation of foods.

1. Most foods will oxidize through a metal catalyzed reaction since metals are present in the necessary trace amounts (1 ppb to 500 ppm).
2. Most likely, a metal hydroperoxide complex is formed before decomposing to free radicals by addition into the coordination sphere of the metal.
3. Metals that are most active usually have a valence state change of $+3 \leftrightharpoons +2$ with the oxidized + 3 state being more active in reduction of the hydroperoxide decomposition activation energy.
4. Competition for the metal by other coordination species may either reduce or raise the oxidation rate by changing the electronic structure of the outer shell of the complex.

5. Certain chelating complexes may reduce the catalytic effectiveness of a metal by steric hindrance even though all coordination positions are not filled.

6. The solvent system has a very significant effect on metal catalysis by:

a. If water, which is the primary solvent of food systems, is present, it may complex with metals preventing the complexing with hydroperoxides.

b. The solvent may react with the metal to form an inert insoluble compound such as a metal hydroxide.

c. The solvent may increase the mobility of the metal catalyst.

Kamiya et al.[72,110] showed that in the cobalt catalyzed oxidation of tetralin, addition of 10% of water by volume reduced the rate by a factor of five. The water was presumed to form insoluble cobalt hydroxides, as well as preventing the metal-ROOH complex from forming, based on the reaction kinetics. Uri[48] showed that metal salts did not catalyze the oxidation of methyl linoleate in ethanol, ethyl acetate, and ethyl caprylate, while in the nonpolar solvent benzene they were very active. By adding increasing amounts of nitrobenzene, a fairly polar solvent, to linoleate in benzene, the rate decreased in proportion to the molar concentration of nitrobenzene that could be present in the coordination shell of the catalyst. Dean and Skirrow[111] found that addition of 3% water to cobalt acetate catalyzed system of t-butyl hydroperoxide caused a 25 fold decrease in rate. It was verified by spectroscopic data that water pushed acetate out of the coordination shell of the metal. Hendry and Russel[112] also found that polar solvents other than water also hydrogen bonded with the hydroperoxides produced during oxidation preventing or at least slowing their further decompositon. Some solvents also form very strong complexes with transition metals to act as good inhibitors.[113] In most cases, water, at least starting with dry systems, acts as an antioxidant in that it reduces reaction rates.[60,114] However, in a few cases,[72,115,116] water acted as a solubilizer or mobilizer for pure systems thereby increasing catalytic effectiveness. The effect of water on food oxidation is an important factor and will be discussed subsequently. A recent Symposium[117] which will be published by SIK should also be of value in a review of recent studies of metal catalyzed oxidation.

ANTIOXIDATION

Types of Antioxidants

Modern methods of food processing and handling require the addition of certain chemicals in order to insure high quality and a shelf life equal to, if not greater than, the normal distribution and marketing time of foods. Antioxidants, as a class of chemicals, are ubiquitous in their presence by addition to foods including cereals, bakery products, snack foods, animal feeds, intermediate moisture, and dehydrated foods, as well as incorporation into packaging materials. They do not improve the quality of the product, but, rather, maintain it by preventing oxidation of labile lipid components. They also are effective at very low concentrations, as would be expected for inhibition of a free radical chain reaction.

To prevent autoxidation from occurring, or at least to minimize it, several types of antioxidant methods can be utilized. Scott[118] has definitively reviewed the whole area of antioxidant mechanisms and classifies them into three types:

Type I free radical chain stoppers, of which BHA (butylated hydroxy anisole), BHT (butylated hydroxy toluene), PG (propyll gallate), tocopherol, and gum guaiac are examples, in foods. These are primarily phenolic type compounds which can donate a hydrogen to a radical.

Type II - free radical production preventors in foods, such as the chelating agents EDTA (ethylenediaminetetraacetic acid), citric acid, and various forms of ascorbic acid. These act mainly by tying up metal catalysts.

Type III - environmental factors, such as lowering of oxygen partial pressure in the package or holding at a critical moisture content for a dehydrated food.

Type I Antioxidants

Kinetics

These antioxidants work primarily by breaking the free radical chain reaction through removal of either the alkylperoxy or alkyl radicals from the chain step according to the reactions below:

$$ROO\cdot + AH \xrightarrow{k_a} ROOH + A\cdot \qquad AB.$$

or

$$R\cdot + AH \xrightarrow{k_a'} RH + A\cdot \qquad AC.$$

and

$$A\cdot + X \xrightarrow{k_t''} \text{Non-radical} \qquad AD.$$

where AH is the antioxidant, k_a, k_a' are rate constants, and X is some other moiety. The type of protection afforded is an increase in the induction period and a slowing of the rate during this period. The kinetics of this have been thoroughly reviewed in several articles and books.[4,118-120] Typically what is observed, at least in lipid systems, is represented in Figure 10 for an oil.

In the case where oxygen is not limiting, which could occur in most foods under normal packaging conditions, the reaction with the alkyl substrate radical (AC) can be neglected since it is changed quickly into the peroxy radical. In addition, the reaction is endothermic since the association of R-H provides 80 kcal, whereas dissociation of the hydrogen from the antioxidant moiety requires 90 to 100 kilocalories, depending on the type used. Assuming that one starts with pure material and that all or most of the termination takes place by the antioxidant route the overall reaction rate then becomes:

$$-\frac{d(O_2)}{dt} = \frac{d(ROOH)}{dt} = \frac{k_p\, k_i\, (M)}{k_A\, (AH)}\, (ROOH)\, (RH) \qquad (26)$$

In this case we have also assumed that the antioxidant radical produced is nonreactive, i. e., it does not enter the chain but terminates in some nonradical producing mechanism. In addition, since the lipid or food is relatively pure to start with the initiation is by monomolecular decomposition.

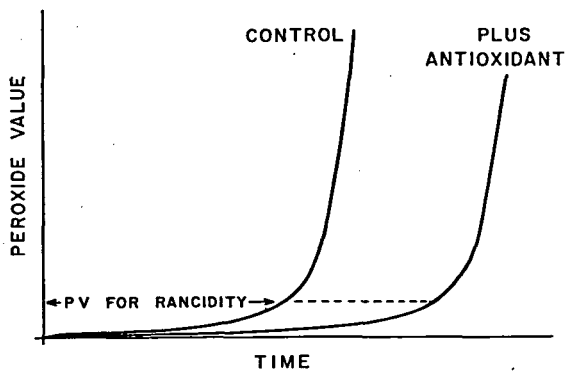


FIGURE 10. Effect of the addition of antioxidant on oxidation extent.

An analysis of Equation 26 shows that as one increases the antioxidant concentration, the overall rate should decrease proportionally. Assuming that the amount of substrate utilized is small, then the rate becomes:

$$\frac{d(ROOH)}{dt} = K_A \frac{(ROOH)}{(AH)} \qquad (27)$$

where

$$K_A = \frac{k_p\, k_i}{k_A} (M)\, (RH) \qquad (28)$$

Integrating this between the zero time peroxide value and the value at the time of rancidity or the end of the "induction period" where:

$(ROOH)_o$ = initial peroxide value
$(ROOH)_I$ = peroxide value at rancidity

and assuming the amount of antioxidant utilized during this time is small, so AH is relatively constant then:

$$\log_e \frac{(ROOH)_I}{(ROOH)_o} = \frac{K_A}{(AH)}\, \theta_{IND} \qquad (29)$$

where θ_{IND} = stability time or time at end of induction period. Thus, a plot of the log (peroxide value) vs. time should be a straight line, and the slope a measure of the rate. Unfortunately, this has never been applied to the study of antioxidant effectiveness because of the difficulty of measuring low extents of reaction. In most cases the typical methods utilize some measurement of an end point such as the AOM test, acid test, or weight gain test.[121,183] This is subject to criticism since the peroxide value or weight change may differ at the rancid endpoint for different antioxidants as well as for different temperatures.

Equation 29 also shows that the protective factor or induction time is directly proportional to antioxidant concentration. This is true up to a point. However, the reaction of the antioxidant with peroxides also is occurring, but very slowly by:[118]

$$ROOH + AH \longrightarrow RO\cdot + A\cdot + H_2O \qquad AE.$$

As one increases AH beyond a certain limit this reaction is enhanced and protection no longer occurs, or is actually diminished.[60] PoKorny[183]

has reviewed much of the European work in this area. The effect of increased antioxidant level is shown diagrammatically in Figure 11. A second limitation of the addition of Type I antioxidants is the time of addition. If the peroxide level is too high, the antioxidant effectiveness is virtually masked. This is also illustrated in Equation 29 which says that when $ROOH_o$ is equal to, or greater than the peroxide level at which rancidity is observed, no induction period is observed and no effectiveness occurs. There have been many reports of this when antioxidants were dumped into oxidized olefin systems with no effect occurring.[118,186] As in the cases of food quality, once the product is rancid, addition of acompound cannot make it better again. PoKorny[183] also has evidence that phenolic antioxidants offer very little protection if the metal concentration is too high.

When oxygen is limiting, such as in good vacuum packaging with a level of available oxygen less than 0.5%, then a synergistic effect occurs. This is illustrated in Equation 30 which is the solution for this condition:

$$-\frac{d(O_2)}{dt} = +\frac{d(ROOH)}{dt} = \frac{k_o\, k_i\, (M)}{k_{A'}\, (AH)}\, (ROOH)\, (O_2) \qquad (30)$$

In this case, lowering the oxygen level shows a direct effect on the rate of oxidation in synergism, with increasing the antioxidant level.[180]

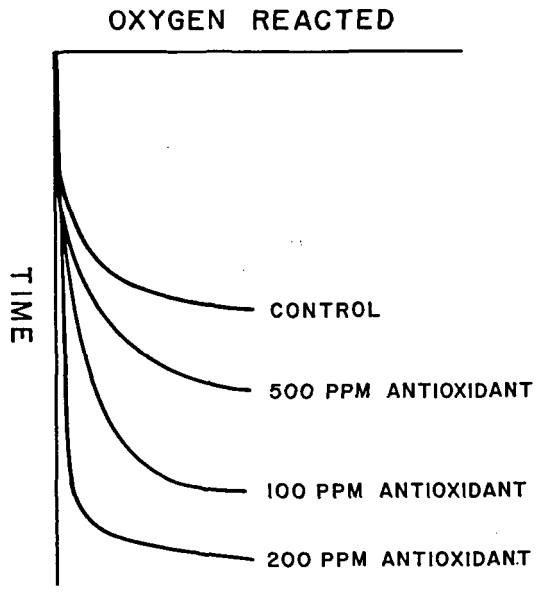
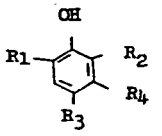


FIGURE 11. Effect of increasing antioxidant concentration on oxidation extent.

For foods the major Type I antioxidants are those which are hydrogen donors of the phenolic type:



Scott[118] has thoroughly reviewed the rules of substitution at the ortho, meta, and para positions in terms of antioxidant stability. Basically, to be a good antioxidant, the compound must be able to have an effective delocalization of the unpaired electron produced in the reaction with the free radical. The more effective the resonance forms, the better the antioxidant. Therefore, substitution at R_1 and R_2 (ortho) and R_3 (para) are much more effective than at the meta R_4 position because of the greater number of resonance forms possible. Another important factor is the size of the substituting group. A bulky attachment helps to protect the antioxidant radical and gives more stability towards further reaction, but this also makes it more difficult to react with the peroxy radical.

Lastly, the reaction of the antioxidant with a peroxide as illustrated above (AE) is fairly temperature dependent. Studies such as the AOM test, which are done at high temperature (100°C) may not correlate very well with results at room temperature.[122,123] This is illustrated in some data shown in Table 7. Hoffman[124] succinctly stated in his review of the analysis of the keeping quality of oils that with most lipids, especially when organoleptic or chromatographic techniques are used to determine the onset of a rancid odor, no general correlation between time and type of off flavor development exists between accelerated storage tests and normal storage. This is especially true in foods.

In conjunction with Type I antioxidants, many feel that there are specific compounds which show synergism, i.e., they increase the induction period by more than just a additive effect.[4,124,125,152] Olcott[125] has described much of this work in this area using squalene as a model, but most of the compounds tested are not suitable for use in foods. Tappel[127] and Uri[4] suggest that

TABLE 7

Antioxidant Testing*
Safflower Oil 0.05% Antioxidant
Time to PV = 70

Sample	AOM test	100°F air
Control	9	21
BHA	9	21
BHT	12	50
PG	24	90
NDGA	12	56
4,4-thiobis (6-6-butylmcresol)	9	113
2,2-thiobis (6-T-butylmcreosol)	8	19
Hydroquinone	39	191
Methyl hydroquinone	69	330
Tetramethyl hydroquinone	9	9
Tert-butyl-hydroquinone	79	372
Catechol	12	25
Methoxyhydroquinone	61	296
Gentisic acid	14	37

*JAOCS *43* 683 (1966)

ascorbic acid works by synergism in the same manner by the following mechanism:

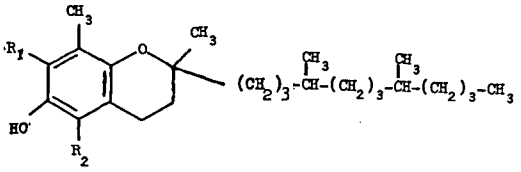
$$AH + ROO\cdot \longrightarrow ROOH + A\cdot \qquad AF.$$

$$A\cdot + BH \longrightarrow AH + B\cdot \qquad AG.$$

It is a requirement that the synergist BH be less reactive with the peroxy radical and form a more stable radical (B·) than the antioxidant radical (A·). Olcott et al.[126] have shown that the antioxidant properties of purified phospholipids are negligible, but that they do have good synergistic effectiveness due to the nitrogenous moieties on lecithin and cephalin. Unfortunately in some cases,[128] the synergistic ability is ascribed to ascorbic acid and citric acid, but they are functioning more as chelating agents. The end result is the same but the mechanism is different.

Type I - Use in Foods

Examples of the common synthetic antioxidants are illustrated in Table 8 for use in human foods. One problem recently studied is the use of a combination of these in salad dressing where partition between the oil and water phase is important and should be considered in food manufacture.[130] BHT, because of its greater resonance stability, is a good synergist in combination with BHA, a common industrial practice. Tocopherol (Vitamin E) in its many forms is a natural antioxidant found in various concentration in many foods, especially plants.[129] Much work has been done with tocopherol because of its presence in the diet and the aspect of aging and oxidation discussed previously. Tocopherol is found in different forms depending on where it is substituted as below:

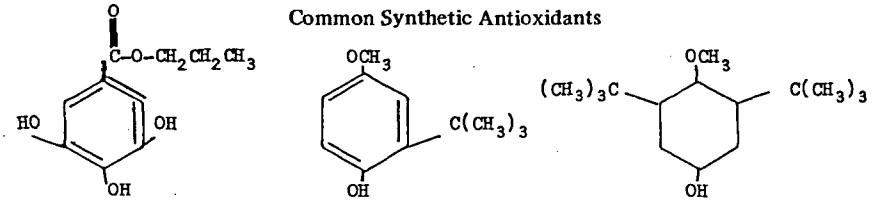


α tocopherol $R_2 = CH_3$ (Vitamin E)
β tocopherol $R_2 = CH_3$
γ tocopherol $R_2 = H$
δ tocopherol $R_2 = H$

The results of the various studies are sometimes confusing as to the effectiveness of each type and

TABLE 8

Common Synthetic Antioxidants



	Propyl Gallate	Butylated Hydroxy Anisole	Butylated Hydroxy Toluene
Name:	Propyl Gallate	Butylated Hydroxy Anisole	Butylated Hydroxy Toluene
Symbol:	PG	BHA	BHT
FDA limit:	200 ppm (fat basis)	200 ppm	200 ppm
Advantage:	water soluble	Fat Soluble	Fat Soluble
		Heat Stable	Heat Stable
			Good Synergist
Disadvantage:	Forms dark salts with iron	Volatile in drying	Volatile in drying
		Off odor	Off odor

will be summarized below.[131-136] This confusion exists in many cases because of the methodology used. For example, the data of Parkhurst et al.[131] are difficult to interpret in terms of the extent of the oxidative reaction.

It has been found that at 50 to 97°C, increasing the tocopherol concentration above 250 ppm has little effect on increasing the induction period, and is less effective above 400 ppm.[131,133,183] This follows the kinetics previously established for a Type I antioxidant, especially at high temperature. Parkhurst et al.[131] found that the efficacy was $\alpha \ll \delta < \gamma$ whereas, α-tocopherol is most effective as vitamin E. Lea,[137] on the other hand, found α to be more effective at lower temperatures and in a more unsaturated and oxidized system. It could be possible that if comparisons are made at too high an extent of oxidation, the order of effectiveness could be reversed. It is impossible to determine from these studies the true extent. In a carefully controlled study, Olcott and Van der Veen[132] find that δ-tocol is better than γ, which should be expected based on the structure of the compounds. They objected to previous workers' results, because previous systems may have been too high in initial peroxide values to give meaningful results. In all results, however, a combination of α with another form gave good synergism as expected from the stereo-configuration of the α compound. Kanno et al.[133-135] present some very interesting work in milk fat where the time to reach a level of 30 meq peroxide/kg fat is a basis for comparison, a value that should be significant in terms of the induction period. As with Type I antioxidants, the effectiveness decreased above a certain level. The effectiveness of the various forms depended on the concentrations used, although at 500 ppm the expected order was found. It is difficult to relate studies in one system to another because the various termination reactions and degradation steps will be different. Kanno found no synergism for α-tocopherol, but he studied this at less than 30 ppm.

One point made in most of these studies is that the relationship between in vivo vitamin efficiency and *in lipid* antioxidant ability is reversed.[136] It must be understood that this is possible since there is no direct proof for Vitamin E acting as an antioxidant in living tissue. Recent work has been centered on reactions of γ-tocopherol with lipid peroxides in aqueous emulsions.[138-140]

Whether tocopherol should be used as a food antioxidant additive is a question of price. Kovats et al.[141] showed that it was quite effective in protecting loss of Vitamin A (β-carotene) in margarine and butter, but its use is minimal since BHA and BHT are much cheaper. Labuza et al.[142] have shown that these latter two compounds are much more effective than the tocopherols in various freeze-dried systems.

Nickerson,[143] Emanuel' and Lyaskovskaya,[119] and recently Stuckey[144] have reviewed the methods of use and the Food and Drug Laws in relationship to the addition of these compounds to foods. In most foods, Type I antioxidants are limited to less than a total of 200 ppm based on the fat content or to a specific level on a dry solids basis. Numerous workers have shown that this is below the toxic level for humans (see reviews by Stuckey,[144] Reimenschneider,[145] Schultz,[1] Lundberg,[120,146] and Emanuel.[147] Although felt to be less effective, the Type II additives such as citric or ascorbic acid, present no toxicological problems because of their natural presence in food and their presence in normal metabolic processes. However, this is not the case of EDTA, which is fairly toxic. Nickerson[143] has compiled a listing of all antioxidants, their use level, and the FDA limits as to toxicity.

Antioxidant Effectiveness

The major question of vital concern to our society is whether some of these chemicals are necessary or effective in preservation of susceptible foods by slowing development of rancidity. This question has been raised before from the standpoint of public safety and moral grounds.[148] If the nontoxic compounds can be used more effectively or better control of environmental factors through processing and packaging can prolong the storage life of food, then use of these compounds could be eliminated.

That this is a question of major concern has been pointed out many times. Reimenschneider[145] stated, after thoroughly reviewing the whole field of antioxidant use up to that time (1955), that there was a very poor correlation between the effectiveness of antioxidants found in whole fat substrates compared to the protection afforded when these fats are in foods. He felt that some progress was being made, but results in most cases were inconclusive and sometimes disap-

pointing. Matz[149] concurred with these thoughts in relationship to the bakery industry where antioxidants have found some success. It was stated that no simple direct relationship or correlation could be made between the various accelerated tests and actual storage life and that more work was needed on this. In addition, Talburt and Smith[150] feel that, although antioxidants could control rancidity development, especially in potato chips, more improved and less expensive methods of control of oxidative deterioration were the most important needs of the potato granule industry.

This leads to the important question of whether the methods by which the effectiveness of antioxidant compounds have been proven and tested in foods are useful and reliable, a question which was put forth to the food industry by Lea in 1958.[151] Thompson[122] felt that present antioxidants are not effective and better but similar phenolic type compounds could be synthesized. His paper points out the fault of much data on antioxidant effectiveness. In most cases, the effectiveness of present antioxidants has been measured in fats or oils by the AOM (Activated Oxygen Method or Swift Stability Test) procedure, the Schaal Oven Test, or an oxygen bomb procedure.

All three procedures involve oxidation at high temperatures (65 to 100°C) in order to be able to test fat or food stability quickly. In many cases, using pure oils and fats, excellent data can be collected on antioxidant effectiveness by these techniques. However, in foods, especially those low in fat and high in protein content, testing at temperatures higher than normal storage conditions causes anomalous results. McWeeney[153] has recently reviewed the area of negative temperature coefficients for lipid oxidation in foods. Due to competitive reactions; reactions which in themselves produce antioxidants at high temperatures (e.g., nonenzymatic browning); change in mechanism pathways; and thermal destruction of reaction intermediates; in many foods, studies at high temperature have no relationship to actual storage stability. That this is evident in rancidity testing of foods was shown by Ottaway and Coppock[154] for biscuits and by Karel and Labuza[13,31] for freeze-dried meats and vegetables, among many other investigators.

This can also be observed in pure lipids as in the data of Thompson[122] for phenolic antioxidants and for tocopherols addition to methyl linoleate.[137,151] Sherwin[121] recently reviewed the methodology of measurement of antioxidant effectiveness and concluded that most techniques used, such as peroxide number, or the TBA value using the AOM procedure, present difficulties in interpretation of stability in comparison to an actual shelf life study or to the more complicated Warburg oxygen absorption measurement as was pointed out as early as 1941 by McKinney.[156] The oxygen absorption procedure gives a direct measure of extent of rancidity development, whereas, the TBA test has value only as an empirical measure.[184] Quencer et al.[157] have shown that for oils, Warburg measurement is the best method for antioxidant evaluation. This technique has only been recently extended to some studies in actual foods.[10,13,31,155]

In real foods, the fat is usually a minor component and the other constituents have a strong influence on the mechanism of oxidation. Because of this, most research on antioxidant effectiveness has been carried out in pure fats and oils under accelerated conditions, whereas fewer studies have been done on actual foods. Riemenschneider[145] has reviewed the major works on effectiveness of antioxidants in vegetable and animal fats. In summary, the phenolic antioxidants (Type I) give very good protection in animal fats, most vegetable fats contain high enough amounts of tocopherol to be fairly stable, and the addition of chelating agents in combination with Type I antioxidants insures extremely long shelf life. More recent works have confirmed these results, and in most cases it was felt that organoleptic evaluation, although the final test, could only be used qualitatively, whereas some chemical methods of rancidity measurement afford a more easily determined index of rancidity. Emanuel and Lyaskovskaya[119] have made a more recent review of the use of antioxidants and list over 600 references which confirm these results. Chipault[148] also includes over 350 references with the same conclusion. In summation, many Type I antioxidants give excellent protection in unsaturated fats and oils (especially at room temperature) and afford fair to good protection in most baked goods, cereals, nuts, and milk powder, increasing the shelf life by 15 to 200%.

In many of these studies, however, the amount of data collected and the evaluation of the onset of rancidity are questionable. It is reasonable to

review a few results which are typical of both good and questionable effectiveness. Very early work was done at the American Meat Institute Foundation in Chicago on improving the stability of lard for use in foods. Kraybill,[158] using the Schaal Oven test (holding at 65°C and smelling sample periodically), found that BHA and PG were excellent for improving the stability of potato chips fried in animal shortenings and for pastry made with lard. However with vegetable oils Magoffin and Bentz[159] found only 5 to 30 days improved storage life for chips. Neumer and Dugan[160] found that the Schaal test lacked precision in testing dry dog foods stabilized with antioxidants and measured volatile carbonyl production instead. This latter test showed that some antioxidants were effective, but the test was carried out at 100°C. Dugan et al.[161] showed an increase of three weeks for the shelf life of dog biscuits and corn chips using BHA to which hydroquinone was added. An organoleptic evaluation was used at weekly intervals.

In animal feeds, BHT and BHA increased stability by four to six weeks as observed organoleptically and by loss of vitamins.[162] Similar improvements have been found for corn, oat, and rice cereals held at 74°F by Stuckey[163] and Anderson et al.[164]

In most of these tests, very few data points were taken, especially if long term storage was used higher than approved levels of antioxidants or nonapproved Type I antioxidants were used, and accelerated storage temperatures were used. In any case, however, as has been found by extensive practice, some improvement by the use of antioxidants if afforded to baked goods, nuts, animal feeds, and whole milk powder; all cases being foods of high fat content or with added animal fats. The Quartermaster Food and Container Institute for the U.S. Army did extensive long-term storage studies of baked goods during and after World War II to confirm these results.[165]

The results of more recent work on foods of low fat content have been rather disappointing, although Type I antioxidants are still used quite extensively. Drazga et al.[166] found that nitrogen packaging (i.e., exclusion of oxygen) was as good or better than the addition of BHA, BHT, or tocopherols for potato flakes. In this case, they used an organoleptic taste panel for rancidity evaluation and the study took over ten months. Gooding,[167] in reviewing the stability of dehydrated foods, felt that the only practical method of oxidation prevention was either nitrogen or vacuum packing. With sweet potato flakes, Deobald and McLemore[168] found that antioxidants were only effective when the oxygen level was at about 10%, whereas, above or below this value, little extra protection was evidenced. In this study, which took two years, carotene loss was used as the index of stability. Abbot and Waite[169,170] found little protection offered by tocopherols for dry whole milk powder whereas PG and BHA were somewhat effective. They followed rancidity by determining peroxide values at monthly intervals over a one year period. That peroxide values are not indicative of oxidation level in dehydrated foods has been pointed out previously.[10,148] In these latter cases, Warburg oxidation was the recommended procedure for good correlation with rancidity. Chen[171] used a baking test for measurement of antioxidant effectiveness in dried yeast and found some protection in a study lasting only five weeks; however, normal storage would be much longer than that and so the results could not be used for practical purposes. Other papers showing both positive and negative results are compiled in the extensive reviews referred to earlier.

In summation, results in dried food systems have shown variable results as to antioxidant effectiveness and the methodology used for testing effectiveness is not consistent with the present knowledge of oxidation kinetics. Since antioxidants are being continuously proposed for addition to all types of foods including the newer intermediate moisture type, more rapid and realistic techniques for determining their relative effectiveness over packaging at low oxygen levels must be found. These recommendations are consistent with the findings of the panel on the current important problems to be solved in food science and technology.[172]

Type II – Antioxidants

As described previously, other mechanisms exist for controlling the rate of oxidation. Those agents which affect the initiation rate, i.e., control the source of the production of free radicals before the propagation step are classified as Type II antioxidants. The major Type II antioxidants for food use are the chelating agents which coordinate with trace metals making them less reactive.

Metal Ion Deactivation

Trace metals present in food or lipid systems reduce the activation energy for initiation. This tends to increase the rate at which oxidation occurs and is of importance to the food industry in the removal or inactivation of these metals. This is of particular importance in the salad and cooking oil industry.[173]

The overall catalytic activity of a metallic ion is controlled by the slower step of the redox cycle below:

$$M^{+2} \underset{\text{slow}}{\overset{\text{fast}}{\rightleftharpoons}} M^{+3} + e^- \qquad AH.$$

It not only depends on the electronic structures of the metals themselves but on the solvent system and ligand (coordinated) groups attached to the metal. Σ donor ligands, such as amines, stabilize the oxidized state thereby increasing the reaction rate of direct radical formation. The reverse is generally found with π acceptor ligands such as the heterocyclic bases. These compounds, such as hemes, stabilize the M^{+2} state but also increase the rate of peroxyl-chelate complex formation thus also increase the rate of radical formation.[89]

No known attempt has yet been made to use this electronic structure chemistry to understand catalysis in food lipids. So whether a compound works or not to reduce activity, is an unknown factor. Ethylenediaminetetraacetate (EDTA) is used for chelation of many metals and seems to be an excellent oxidation inhibitor in foods, especially those of intermediate and high moisture content.[142] EDTA, however, is limited to low levels of addition (in some foods less than 100 ppm on a dry solids basis).[144] Another heterocyclic compound like EDTA is 1 to 10, o-phenanthroline which is a very strong chelating agent with iron for which it is used in micro-analytical determination.[174] However, it activates the iron, making it more catalytic then free iron. At equal molar concentration the rate of oxidation for o-phenanthroline with iron was eight times faster than for EDTA with iron, which was twice as slow as the control.[175]

Citric acid in various forms such as isopropyl citrate is another very popular chelating agent,[173] especially for oils and in intermediate moisture foods.[142] It is very pH dependent on its activity in a food. Ascorbic acid is also felt to work as a chelating agent in dry systems but the mechanism becomes more complex in the presence of water due to the formation of radicals by itself.[103-105] It is too difficult in food systems to separate the chelating effect from the Type I synergistic effect as proposed by Privett.[176]

Amino acids have also been implicated as having some chelating ability and indeed can either increase or decrease the rate depending on the system and metal.[177-179] Their addition is limited, however, due to solubility and FDA approval. In fact, all antioxidants incorporation into a tissue foods is a real problem. The amino acids, however, deserve further study as Marcuse[180] has found significant synergism with reduced oxygen levels. One last problem which relates to accelerated storage studies is that most chelate complexes are less stable at higher temperature. Therefore, the testing must be done at normal storage temperature to insure adequate effectiveness.

Ultraviolet Light Deactivators

Certain compounds can react specifically with ultraviolet light to produce very stable radicals. These could be used to reduce UV induced initiation and many compounds are utilized in the rubber and plastics industry.[118] Unfortunately, they are essentially toxic and cannot be used in foods. In addition, with most foods the penetration of UV light beyond a few mm depth is negligible so that this type of antioxidant is not useful. One compound that could be used is carbon black, as it is the most effective, but its color would make a food unacceptable.

Type III – Antioxidants

The Type III antioxidants are not really associated as the addition of specific compounds to slow the reaction, but rather as the control of environmental factors. For example, reduction of the oxygen level has a definite antioxidant effect as expected from the kinetics. In addition, other environmental factors such as temperature and moisture content also affect the rate. The kinetic aspects of some of these factors have been covered already, and will be further discussed in terms of food storage in the following sections.

THE FOOD ENVIRONMENT AND LIPID OXIDATION

Overall View

Up to this point the review has been concerned

mainly with the basic kinetics of the free radical reaction as occurs with pure lipids. It is justifiable to state that the reaction is much more complex in a food. Foods contain many components other than lipids that can affect the rate of oxidation such as metals in the heme form, free amino acids, proteins, and natural antioxidants. This makes the kinetic analysis of oxidation reactions extremely difficult as one cannot account for all the interactions. Figure 12, for example, illustrates the fact that foods such as beef or fish do not follow the typcial reaction scheme as does the oxidation of potato chips.

Other fractions of the food system can react with oxygen, making measurement of oxygen uptake erroneous. Tappel[187] has shown that with meat fractions the lipids may account for only 10% of the total oxygen reacted. Some of this nonlipid oxidation is found to occur directly with proteins.[60,187]

The oxidation of foods can be categorized into the effect of:

1. surfaces
2. nature of the lipid
3. proximity of other compounds + components
4. solvent system i.e., presence and amount of available water.

Effect of Surfaces

A trend in the study of lipid oxidation in foods is to use model systems comprised of some solid surface over which the lipid is dispersed either in the dry state or in emulsion. This allows for the study of the interaction of various components with the idea of scaling up the findings to real foods. Koch[188] has summarized much of the earlier work of model system study done by the Army laboratories.[189–191]

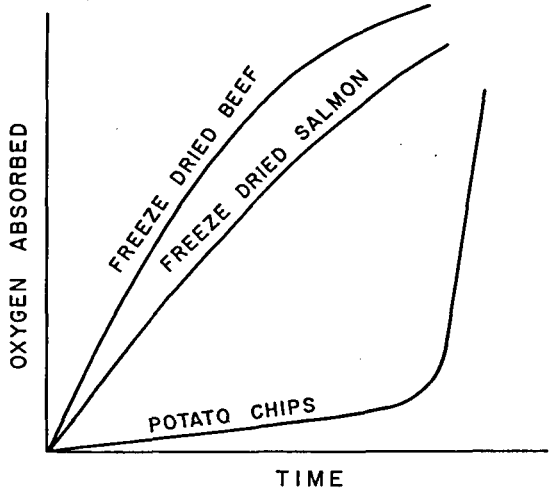


FIGURE 12. Typical oxygen absorption curve for oxidizable foods.

In the study of soybean oil dispersed on various surfaces, protein type surfaces always inhibited the reaction rate with gelatin the most effective. Carbohydrate surfaces gave the fastest reactions. The reasons could be due either to orientation of the lipid protecting it in some way or interaction of the surface with radicals to take them out of the normal pathway.[181,182] In addition, the trace metal content of these sytems may have been vastly different accounting for the effect. Using purified linoleate, Labuza et al.[60] found that egg albumin decreased the oxidation rate by almost 100 times in very pure systems, suggesting that the protein-lipid reaction may be the important one. It has also been reported that some surfaces such as Teflon® could catalyze peroxide breakdown to free radicals giving an accelerating effect.[192] The juxtaposition of various surfaces can either increase or decrease the reaction rate, possibly by orienting the lipid.

The lipid structure itself has some influence since the way it sits on the surface of a solid phase can effect its contact with oxygen. Generally triglycerides react more slowly (about eight to ten times) than esters which are slower than the free acids.[60,185] Randomizing the distribution of fatty acids in natural lipids significantly decreases the rate of oxidation by putting a larger quantity of the unsaturated fats in the 2-position on the fat.[193] Badings,[298] however, reports no differences for the oxidation rates of different forms.

An additional interesting factor affecting oxidation is whether the lipid is in the solid or liquid state. Several studies have shown that the rate of oxidation of a lipid is much higher than expected when in the solid phase.[153] Betts and Uri[194] feel this is because in the solid phase the ROO· radical, being slower to diffuse, reacts more readily with the substrate continuing the propagation, whereas the radical in the liquid fat can terminate more readily with other radicals. This could explain the poor stability of many frozen fatty foods such as chicken and meat.[195]

Proximity of Other Components

As previously stated, foods contain many natural components that may affect oxidation rates. The effect of metals and various antioxidants have been discussed. In a real food it would be difficult to assess the individual affect of each, so most studies are made in model systems. This, in turn, may complicate the conclusions because of the way in which the materials are combined. Although phospholipids are described as being antioxidants,[189] Corlis and Dugan[196] have shown them to oxidize very rapidly with rates and activation energies close to that of methylesters. Prior work was done with very impure mixtures, probably accounting for the opposite results. Buttery et al.[11] show that the phospholipids are the first to oxidize in dehydrated potatoes, which should be expected. Some antioxidant effect can be attributed to the nitrogen group on the phospholipid but this needs further work.

Free carbohydrates have also been implicated in increasing oxidation rates in emulsions.[186,197] This is attributed to either a co-oxidation of the sugar or an additional catalysis of peroxide decomposition. How important this is in an actual food is not clear, but certainly in the new type of emulsion-convenience sauces and salad dressings the levels of sugar and oil are high enough for this to occur. This might also be of importance to the stability of sugar cured meats.

Another complicating example is the effect of amino acids as previously discussed. Coleman et al.[198] found histidine to act as a catalyst in linoleate emulsions; however, in a more definitive study Marcuse[178,199] showed that this was pH dependent. At low pH, histidine is an antioxidant, whereas at higher pH it is a proxidant. Tijho et al.[177] showed this also depended on the metal added and the amount of water present.

Other deteriorative reactions can also effect lipid oxidation. Nonenzymatic browning for example produces complex reaction products which inhibit oxidation.[200,201] Antioxidant properties have also been attributed to tripolyphosphates and extracts of plant tissues,[202,203] although the method in which these latter studies were carried out may have influenced the results. In any case, an actual food is a very complex system and very few *"a priori"* conclusions can be drawn.

Solvent System-Water and Lipid Oxidation

Foods exist as an assemblage of solid and liquid components, either free or in compartments held together by various forces. The most overwhelming substance is that of water in natural foods. Its presence is the controlling factor in the stability of a food during storage. Labuza,[204] and Labuza et al.[206] recently reviewed the total picture of the physical properties of water and its control on food stability based on the observations of Duckworth and Smith.[224]

Figure 13 illustrates the water sorption isotherm that is typical of most foods.[205] For any system, including foods, the water activity is defined by Equation 31:

$$a_w = \frac{\%RH}{100} = \frac{P}{P_o} \qquad (31)$$

where

a_w = water activity
%RH = relative humidity
P = vapor pressure of H_2O in food
P_o = vapor pressure of pure water

From Figure 13 it is seen that the water activity of most tissue foods is very close to that of pure water since their moisture contents range from 60 to 95% on a wet basis. This makes them most susceptible to microbiological decay. This can be prevented by heat sterilization, freezing, drying, or other methods. If not preserved in some manner the microbial growth is so rapid that any chemical deterioration that takes place is insignificant. With preservation, however, the significance of chemical deterioration and especially lipid oxidation becomes increasingly important. It is under this criterion that the amount of water and the water activity have

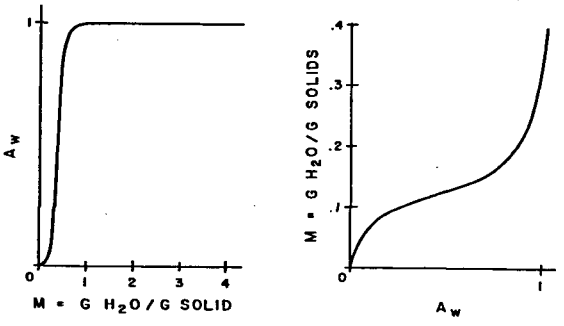


FIGURE 13. Moisture sorption isotherm for a food. Curve on left shows overall isotherm, curve on right shows the isotherm expanded at low moisture content.

an important control over the rates of the various chemical reactions.

In Figure 14, the effect of water activity on the stability of foods is graphically illustrated. What is important to this review is the interaction of moisture and lipid oxidation. The properties of water that are most pertinent to lipid oxidation are:

1. Water acts as a solvent
2. Water mobilizes reactants
3. Water can interact either chemically or by hydrogen bonding with other species.

Historically, dehydration of foods by removal of water, whereby the water activity is reduced to low values, was a good method to prevent microbiological decay. However, it was found that if foods were dried to too low a moisture content (less than 2 to 3%) they became very susceptible to oxidation.[207] It was found that there was an optimum moisture level for dehydrated foods which prevented or at least reduced the rate at which rancidity developed.[208-212] Many theories were developed to explain this phenomenon, but they did not account for the kinetics of the reaction or the physical properties of water. In an investigation of this phenomenon in both model systems and various foods,[10,59,60,213,214] it was shown that the basic protective function that water exhibited when the moisture content increased from the absolute dry state (Figure 14) could be accounted for by two factors:

1. Water interacted with metal catalysts making them less effective through changes in their coordination sphere
2. Water hydrogen bonded with hydroperoxides tieing them up so that they no longer were available for decomposition through initiation reactions.

These two factors slow the rate of initiation, as found from a kinetic analysis of the results. In addition, other work showed that water also caused a rapid loss in free radicals in dehydrated food systems possibly through recombination reactions.[215,216] This shortens the lifetime of radicals immensely, possibly by allowing them to migrate out of the trapped state as proposed by Roubal.[217] The importance of the presence of water is clear if the shelf life of a dehydrated food is to be extended. It should be noted that the dehydration process itself can cause a production of free radicals which could enter into lipid oxidation reactions.[218,219]

One other important theory of how water interacted to slow lipid oxidation was that of Salwin,[212] in which the water attached to sites on the food surface, thereby excluding oxygen from the lipid. This was based on the results of finding an optimum moisture content, close to the monolayer coverage of water on the food. Below this level, oxidation occurred; above it other chemical reactions occurred. Therefore, one would expect that if lipid oxidation was the only possible reaction, the higher the water content or water

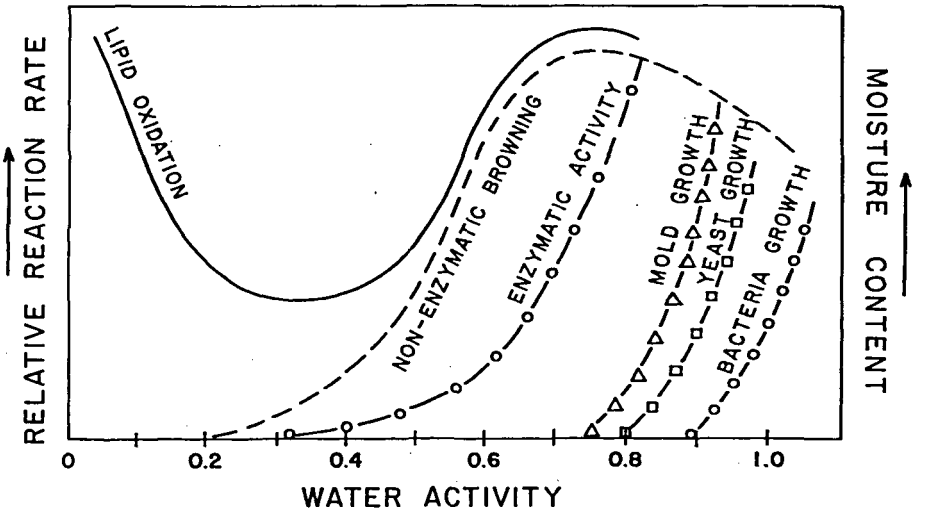


FIGURE 14. Stability of foods as a function of water activity.

activity, the slower the reaction. It was pointed out by Labuza et al.,[60] however, that there seemed to be an increase in oxidation rate above a certain water activity contrary to the protective water layer theory. In a series of studies with model systems and foods[116,142,214,220-223,225] it was found that the oxidation rate increases again to a maximum in the intermediate moisture range or a water activity range of 0.55 to 0.85. Based on this it has been found that the solvent and mobilization properties of water become more important at these higher water activities. The catalysts present are more easily mobilized and possible swelling of the solid matrix exposes new catalytic sites, making oxidation rates even higher than in the dry state. Even more interesting support to this conclusion, is that in a series of experiments in which foods of the same solids, composition, and water activity, but different moisture contents were prepared, the food of higher moisture content oxidized faster.[223] This also supports the much higher rate constants found for methyl linoleate oxidation in emulsions as shown in Table 6. These results indicate that intermediate moisture foods will be very susceptible to oxidation and that foods at their natural moisture content are probably oxidized even faster as illustrated by the short shelf life of refrigerated cooked meats.

Thus, the presence of water seriously affects the rate of lipid oxidation. One factor that has not been discussed is oxidation due to lipoxidase. This will be left to another review as the literature is voluminous and the more recent studies are just beginning to understand the mechanisms involved.[117] It should be pointed out that most dehydration processing should destroy the enzyme, but if it does not, the amount of water present again controls the reaction rate (Figure 14). A thorough review of enzyme activity and water content has been made by Acker.[226]

In the subsequent sections several classes of foods will be examined from the standpoint of their stability to oxidation in light of the above review and especially of the type of rancidity developed in the dry state as compared to the normal moisture state. It is interesting to note, for example, that in a study of oxidation of linoleic acid alone on various metal and glass surfaces, the presence of increasing water vapor in the air atmosphere resulted in a change in the secondary breakdown products.[227] This shows that water also influences the reaction mechanism in some way to produce different end products. It should be expected that this would also occur in foods, making control of moisture content an important criterion in any study. Wilkinson has made the only attempt to try to explain the differences found in food systems based on catalyst concentration, pH, and the phase relationships of the lipids and other solids.[306] He has tried to explain how certain end products such as oct-1-3-one form in the presence and absence of water.

LIPID OXIDATION IN SELECTED FOODS

Beef

The adipose tissue lipids of beef are highly saturated and the lipids of the lean tissues which constitute about 2 to 4% of the total fat are more important in lipid oxidation. These latter are comprised of some phospholipids with close to 25% fatty acids of 2 and 3 double bonds and over 19% with 4 or more double bonds.[228] It is these that are primarily responsible for rancidity development, especially in light of the fact that they are in close proximity with the heme catalysts of the mitochondria.

Raw beef does not present a serious lipid oxidation problem as it is usually degraded under refrigeration by either bacterial or enzymatic action. The basic chemical quality factor is the oxidation of the red oxymyoglobin, and myoglobin, an iron heme pigment, to the brown metmyoglobin but as will be seen this is tied to lipid oxidation. Watts[229] claims that raw meat usually remains below the TBA threshold value of one (indicative of the onset of rancidity) for at least ten days. When an antibiotic was used to inhibit growth of bacteria the TBA score rose to 5.7 in 2 days and was over 12 in one week.[230] However, in this study the meat was ground up so that it presents a different system than the uncut tissue. The meat pigments, which can act as potent catalysts, become intimately mixed with the lipids in this case. The process also distributes oxygen throughout the homogenate, whereas with whole slices oxidation of only the surface might be expected. This is just as detrimental since the rancid surface odor could cause the whole slice to be rejected.

When fresh beef is cooked and stored in a refrigerator the TBA values rise rapidly indicating rancidity.[229] This has been explained on the basis

that the "denatured" form of heme is a better catalyst. However, the evidence for this is very poor and confusing as the electron chemistry of the heme pigment is not clearly understood. Recent studies have tended to confuse the interaction of beef pigments and lipid oxidation.[230,231] Also, all of the work prior to 1960 utilized either chloroform or ether extracts of the meat, only taking out the adipose tissue lipids for rancidity measurements. Even in 1968, in an otherwise good review of the methodology for chemical measurement of beef quality, the role of the lean tissue phospholipids is not considered.[232]

To understand the interaction of the heme pigment in oxidation some knowledge of the electron orbital structure is important. Iron is bound in the porphyrin ring structure (Figure 15) as a square planar chelate. When the iron forms complexes with two additional ligands in coordination positions 5 and 6, this leads to the more stable hexacoordinate octahedral structure, as shown in Figure 16. The iron is free within this structure to be oxidized to the ferric form. In hemoglobin, the 5th position is taken by a histidine attached to a globular protein chain and the sixth position is free (Figure 17). There are four total iron porphyrin rings involved in the structure.

With respect to catalytic activity, the large protein part may in some way cause steric hinderance of the metal, preventing it from catalyzing oxidation. Therefore, when catalase and peroxidase (heme structures) were heat treated, they lost their enzyme activity, but increased the oxidation rate of linoleic acid.[233] In this case it

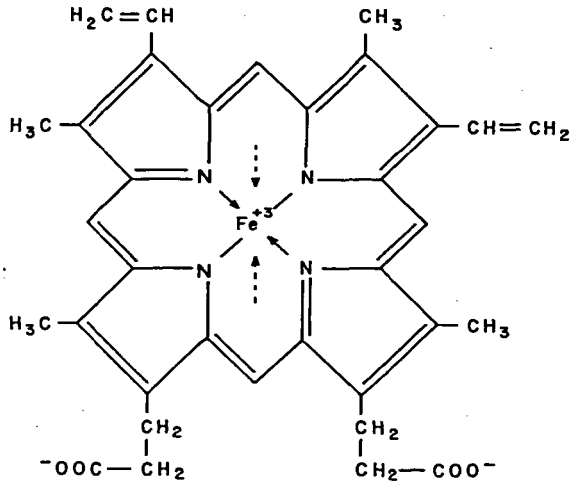


FIGURE 15. Basic structure of a heme compound showing covalent and donor bonds.

was important to note that the iron remained in the ferric state with no valence change. This supports the faster rate of oxidation of cooked meat reported previously. It has been postulated that the protein probably becomes unfolded, allowing easier exposure of the iron to the peroxide. Enzymatically removing the protein portion also increased the catalytic activity of cytochrome C (a heme) by over 12 times,[234] supporting the above. It has been found that the oxidative reactivity follows the order, cytochrome C > hemin > hemoglobin > catalase, which is opposite to the order of the molecular size of the protein. It should be pointed out, however, that heating may be more important in releasing either the pigment or the lipid from a protected compartment, causing more intimate contact. The form of

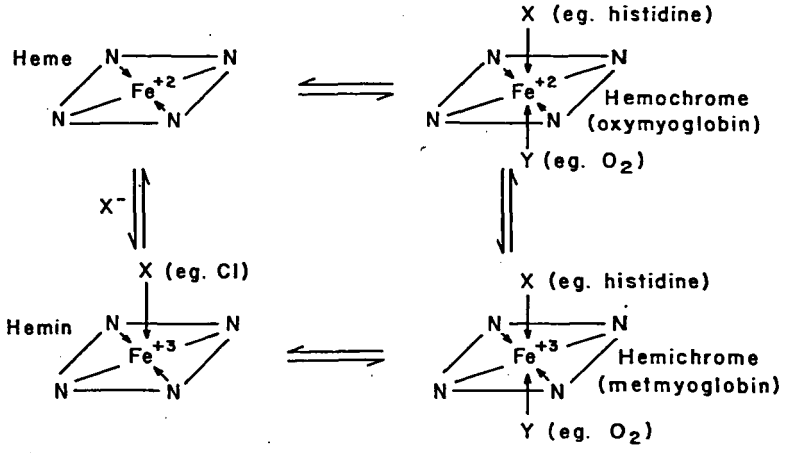
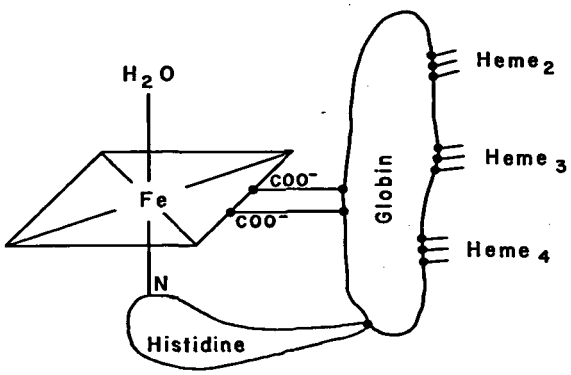


FIGURE 16. Square planar chelate of iron in heme showing the possible arrangements and charge.



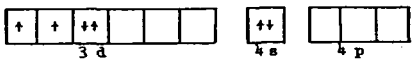
HEMOGLOBIN

FIGURE 17. Structure of hemoglobin.

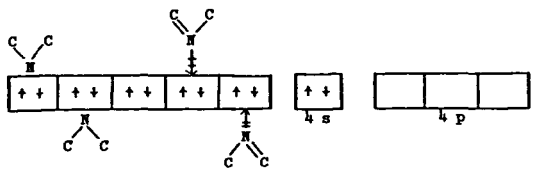
the oxidizable substrate is also important. For example, the acid of linoleate binds with the heme so that inhibition occurs at high heme concentrations, whereas with methyl linoleate, increasing the heme concentration increases the rate.[76]

Based on these above observations, it can be expected that the lipid somehow forms a complex with the heme pigment before it breaks down as was suggested by Tappel.[235,236] Some pigment is also destroyed during oxidation. Thus, its close association in forming the free radicals seems logical, since an oxyporphryl radical could be formed in the homolytic cleavage.[127] This radical could then react within itself or with some other moiety to take the catalyst out of the overall reaction.

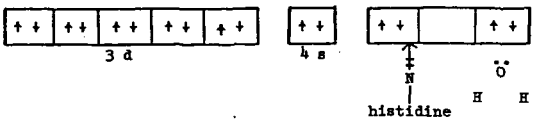
An examination of the outer coordination sphere of the iron complex indicates that association with the peroxide is very likely. Looking at the outer shells, Fe^{++} alone has:



where: ↑ indicates the direction of spin on an electron present. When iron becomes bound to the four nitrogens of heme, two by a direct covalent link and the other two by donation of electrons (a donor bond →) the shells look like:



To get hemoglobin or myoglobin itself we have two more coordinations, the 5th and 6th positions which bring the shells closer to the filled ring of a nobel gas. One is with the nitrogen of histidine from globin and one with water. During oxidation, then, a hydroperoxide complex will push out



the water since thermodynamically a completely filled nobel ring is preferred and the two oxygens in the peroxide can fill the empty 4-p positions. This should give a stable low spin complex. However, since the two oxygens are highly electronegative and are next to each other, a homolytic scission becomes probable given the proper energy transfer from the iron porphyrin ring.

It is in this area of electronic configuration that more information is needed. However, these considerations throw doubt on the contention that the heme compounds have to be altered to act as catalysts as proposed by Watts[237] with an Fe^{+4} intermediate. In addition, Green's[230] contention that oxymyoglobin is not an active catalyst and only metmyoglobin is, is unfounded since most of Tappels work has been with the ferrous form. Her results would seem to indicate that the proposed metmyoglobin is a product of the homolytic cleavage as by reaction (x) rather than the active catalyst form. This is also supported by Williams.[238] Statements in the literature, such as that by maintaining the ferrous state, lipid oxidation is prevented, are incongruous with the theories of lipid oxidation. It is obvious that the ionic state of the metal is not important but that its ring structure is. The methods used to maintain the myoglobin, such as by lowering P_{O_2}, also inhibits lipid oxidation itself. Thus the absence of metmyoglobin does not mean there is no oxidation taking place, as it can easily be reconverted back to oxymyoglobin by several means. The addition of reducing agents, such as ascorbate, probably act by being perferentially oxidized and protecting the pigments and lipids. Brown et al.[240] has also shown that even in simple systems the ferrous form is the more important one and the normal extraction procedure used to remove the pigment can cause the change to the ferric form.

Autoxidation in dehydrated meat is a much slower process than in fresh meat because of the reduced water content; it is the major reaction responsible for deterioration during storage. In a series of studies at the U.S. Army Natick Laboratories it was found that:[241-245]

1. Dehydrated raw beef patties become unacceptable when stored in air for 1 month at 100°F, a much slower rate than for raw beef at 5°C which goes rancid in 2 to 10 days. At lower levels of oxygen, significant protection is afforded. For example, storage life is about 6 months at vacuums less than 20" Hg.

2. Cooked dehydrated beef stew becomes organoleptically unacceptable in 4 weeks stored in air at 100°F, whereas packaging in 30" vacuum affords over 24 weeks acceptability. Chicken stew became rancid in just about two weeks in air and low oxygen. Packaging was not as effective for beef following the characteristic kinetic effects predicted for the rate as a function of P_{O_2}.

3. Using a sensitive oxygen probe, oxidation rates were initially rapid even at 2% oxygen level for chicken, beef, and pork with rancid notes being observed at the 5th, 2nd, and 1st month, respectively. Using a 5% hydrogen atmosphere with a palladium catalyst to reduce to oxygen level to "zero" the storage life was increased to greater than one year but this system is uneconomical and impractical. In commercial packaging usually a 1 to 2% oxygen level is reached for dehydrated foods. This does not limit the rate significantly, but lowers the total available oxygen. Therefore, a low oxygen permeable film is needed. Simon[297] has reviewed some mathematical methods for establishing the proper film.

Unfortunately the data of these studies could not be analyzed to give either K_m or K_B. However, taking the initial rates, the results in Figure 18 were obtained for beef stew, as an example. It is seen that the pattern for oxidation as a function of P_{O_2} follows what would be expected kinetically. The beef stew oxidized most rapidly with other combination items being less reactive. This most likely is due to the amount of labile lipid present but this could not be ascertained from the reports. An analysis of the data of Speiss[246] shows a similar pattern for freeze-dried cooked beef using just the loss in hedonic value as shown in Table 9.

Chipault of Hawkins[247] utilized Warburg oxidation at 60°C since other chemical techniques were found to be inadequate, such as the TBA value. For beef the oxygen absorption curve resembled that shown in Figure 12. This indicates that maximum rate kinetics were probably taking place. Oxidation was followed for over a 30 day period. Oxygen absorption initially was about 20 to 30 μl O_2/g beef/day similar to that for the beef stew (Figure 18). Dried chicken oxidized about two to three times faster, probably because of the higher content of unsaturated fats.

The possible explanation for the unusual oxidation curve found is that other components may be oxidizing, especially since the measured peroxide value was about ten times lower than the oxygen reacted. A square root plot of their peroxide data follows a straight line as could be expected. Since the molar amount of oxidizable fat is unknown, no measurement of K_m could be made. Upon fractionation of the beef, it was found that the protein bound lipids (probably phospholipids) accounted for most of the oxygen absorbed, whereas the free protein absorbed only a little oxygen. This suggests that the low peroxides may be due to the fact that when produced they react rapidly with the protein to pigment which is in close proximity to the oxidizing fat. It is also found that the solubility of the protein decreases rapidly in storage.[239] Maximum rate kinetics are probably applicable for meat oxidation. Similar results were reported for the chicken.

Another factor worth mentioning is that in all these studies oxidation and development of rancidity takes place slower than for the fresh tissue. This is at first unexpected since the dried

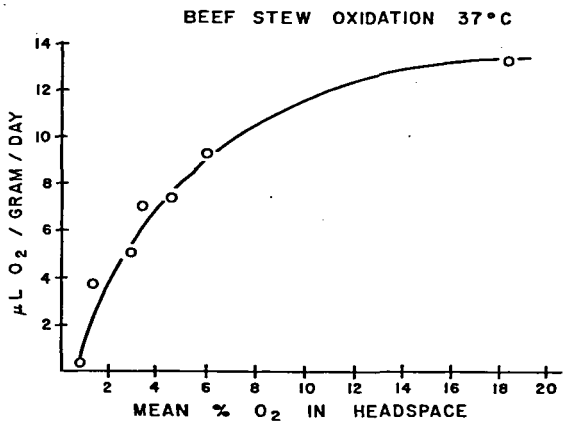


FIGURE 18. Calculated initial oxidation rate of dehydrated beef stew as a function of oxygen concentration.

TABLE 9

Effect of Oxygen Level on Organoleptic Value of Freeze-Dried Beef at Room Temperature[246]

% O_2 in atmosphere	Hedonic units lost in 30 days*
0.1%	0.7
1.0%	1.3
2.0%	3.8
21.0%	5.1

*Initial value = 6.6

product has a larger surface area for exposure of the fat directly to oxygen, and drying itself produces free radicals. Also, one could expect oxygen diffusion to be more difficult in the watery tissue. The key to the difference has to lie in the presence of water, which at high concentration must allow the catalysts to be more mobile and reactive than in the dry state where water exhibits a protective effect. No reports, however, exist as to the differences in the secondary products produced in the dry vs. the wet state. One point not made previously is that it is difficult to compare results between researchers because the tissues may differ due to age, sex, diet, etc.

Other Meat Products

Other meat products behave similarly to beef. However, since the iron heme pigment is at a lower concentration, its co-oxidation is not as important from the color standpoint.

Chicken oxidized more rapidly than beef because of the higher unsaturated fatty content especially as phospholipids. Cooked chicken becomes unacceptable after about four days in refrigerated storage with the dark meat (more phospholipids) having a lower flavor score and twice as high a TBA value.[248] Frozen storage for the same length of time retained the original flavor. Turkey shows a more rapid development of rancidity.[248] This is due to the larger amount of phospholipids in turkey (about 30 to 40% of total lipids.[249] Acosta et al.[249] report Warburg oxygen absorption for turkey lipids at 40°C. They find an unusually high rate of 500 μl O_2 absorbed per hour for the total turkey lipids which reduces to 15 to 20 μl O_2/hour per gram of turkey tissue. This is about 20 to 30 times faster than for freeze-dried beef or chicken. It was shown that this was mainly due to the extremely high rate of oxidation of the phospholipids fraction. This rate is also similar to the maximum rate derived from the data of Labuza et al.[60] in the rapid rate period and close to the rate reported by Badings[298] for trilinoleate and trilinolenate. In all cases, the lipids from cooked turkey oxidized faster than for uncooked tissue, as with beef.

With freeze-dried turkey stored at various moisture contents it was concluded that lipid oxidation was not responsible for deterioration.[250] However, oxygen absorption occurred at a fairly rapid rate, if the data are analyzed as above showing:

at:	
37°C	150 μl/gram/day
20°C	60 μl/gram/day
0°C	9 μ1/gram/day
-20°C	3 μl/gram/day

This is again much slower than in the wet state, supporting the same conclusions as for beef. The authors concluded that since TBA did not increase, the lipids were not oxidizing. However, from an analysis of the oxidation rates, as well as their measurement of the increase in lipid browning, (probably due to oxidative polymerization) an expected activation energy of 14 Kcal/mole is found. In a further study they tried to explain their conclusions based on the orbital theories presented previously for the iron porphyrin complexes.[251] They present some interesting results, but again they base their observations on the TBA number showing no oxidation although the Warburgs show significant oxygen absorption.

Pork also contains a high phospholipid content (~ 20%) of which linoleate contributes 30% of the fatty acids.[252] In addition, the total lipid has about 50 to 60% unsaturated fatty acids. Poor data exist to make a comparison, as with beef and turkey, between the natural and dried state. Zipser[253] studied oxidative changes in cured and uncured frozen cooked pork. Some unusual results are found which are contradictory. TBA values were used as the basis of comparison which might account for the difficulty.

With respect to nitrite, the authors report that during curing the meat pigments are converted to the catalytically inactive ferrous nitric oxide form as also reported by Watts.[254] They concluded that

this is the reason for the higher stability of refrigerated cured pork compared to uncured meat. However, Brown et al.[240,255] finds all forms of the pigment including the nitric oxide form to be catalytically active in Warburg studies with pure lipids. Ellis et al.[256] also reports that the addition of sodium nitrite catalyzes oxidation of frozen cured beef. This is also supported by the orbital considerations presented previously. No other reasonable explanation can be given except that somehow the addition of salt (NaCl) in the curing brine may affect the rate by changing the water activity. This is especially true since in the frozen state, cured pork is much less stable than uncured pork.[253] The salt concentration affects the water activity by Raoult's Law. In the frozen state the food is seen to be at an intermediate water activity where change in concentration of dissolved solutes is important.[204] Further work with pork is needed.

Fish

Fish Lipid Composition

Although most commercially marketable fish have a low fat content, the degree of unsaturation is much higher than in meat, making it much more susceptible to oxidation (Table 1). Ocean fish are more susceptible than fresh water fish, as they have a larger amount of the more unsaturated C_{20} and C_{22} fatty acids on the average.

In lean fish, such as cod and haddock (.5 to 1.1% lipid), 65% of the total lipids are found intracellularly as phospholipids intimately associated with muscle protein.[262,264] The remaining 35% is neutral lipid. Variations in the phospholipids irrespective of tissue or species, are responsible for the most substantial differences in fatty acid compositions.[265] Depot fats for lean fish are found in the liver and peritoneum.

Fatty fish (5 to 25% lipid) such as herring, mackerel, pilchard, etc. have depot fats as extracellular globules in the muscle and in the mesentery. A much higher proportion of the total lipids are neutral lipids. Semi-fatty fish (2 to 5% lipid) such as sole, whitefish, and flounder are intermediate, having depot fat in both muscle and liver, with a substantial proportion of neutral lipid.[266,267] Whitefish, e.g., has a lipid content of 3.84%; 75% which is triglyceride.

Seasonal variations in lipid content and susceptibility to oxidation arise primarily from availability of food and stage of sexual cycle or maturity. Lipid content remains relatively low until sexual maturity is reached. Maximum content and minimum susceptibility to oxidation occurs during post-spawning periods of heavy feeding.[266-269] In cod, lipid content approaches 1% at this time; lipid content of herring approaches 20 to 25%. Halibut, on the other hand, has 0.75% lipid (wet muscle basis) in June and 1.05% in December, reflecting differences in spawning cycles.[270] For Grand Banks and Nova Scotia cod, and high-lipid period extends from June through August; their spawning period being in April and May. Strait of Belle Isle cod, and fish from more northern waters, spawn later, so their period of peak lipid content is late August.[268]

Muscle differences within fish also affect oxidative characteristics. Belly flap tissue is generally richest in lipid and is especially prone to oxidation in the presence of oxygen, as when the fish is gutted.[270] Dark muscle (1.8 to 2.2% lipid) has a greater concentration of heme compounds, less water, more metabolites, glycogen, and soluble precipitates than white muscle (.6 to .75% lipid). The primary function of dark muscle is motive power so it depends on aerobic oxidative processes for energy. White muscle, on the other hand, is the power reserve for rapid movements and derives its energy from anaerobic processes.[271]

Muscle in the tail section contains 25% more lipid than muscle from the head or center section, and a higher proportion of dark muscle, so it oxidizes more rapidly than white muscle from the head. Tail lipids are closely associated with motion, and change very little during spawning; this may be one explanation for the high susceptibility of fish flesh to oxidation during spawning.

Fresh Fish Storage

Under normal conditions of storage (i.e., refrigeration), even fatty species do not become rancid until after putrefaction occurs.[272,273] In the presence of oxygen, though, rancidity will develop, the extent depending on the factors just discussed – type of fish, maturity, season (metal, lipid, and tocopherol levels), and the section of fish – plus, P_{O_2}, and temperature.

At 25°C the major deteriorative reactions are microbial spoilage, leading to production of volatile acids and tri-methlyamine, hydrolysis of protein, and glycolysis.[258,274,275] In the latter, acid (mainly pyruvic and lactic), end products

accumulate and decrease the pH, ATP is degraded to hypozanthine, and amino acids build up. At 10°C proteolysis lags behind other changes; low, but measurable, peroxide values may be obtained for lipid oxidation. At 0°C, fat oxidation becomes significant, but still does not precede proteolysis or carbohydrate fermentation (which is being inhibited by products of proteolysis). Rancidity becomes particularly important only at temperatures below 0°C as will be discussed later. Bacterial growth at temperatures above 0°C has been found to inhibit lipid oxidation though it does not affect final extents of rancidity.[258] Addition of antibiotics or a low radiation dose allows rancidity to develop since growth is suppressed.[257] Lipid hydrolysis to free fatty acids and glycerol is usually predominant, and under appropriate conditions, even lipids of lean fish will oxidize.

The porphyrin compounds in fish exhibit pro-oxidant effects similar to those observed in other biological systems, with corresponding pigment degradation and production of carbonyl compounds.[263,272] Examination of the nine fish species listed in Table 10 showed hematin contents varying from 5.4×10^{-5} M in pilchard to $.1 \times 10^{-5}$ M in cod.[272] Rates of oxidation correlated directly with these contents. The rates of oxidation (Table 10) as calculated from the data are similar to that reported for the other tissues discussed previously but slower than turkey. The data are also in line with the rates of pure linoleate oxidation in catalyzed systems.

The Fisheries Research Group at Halifax has done a large amount of work on susceptibility of fish to rancidity.[258-261] Basically they used fish tissue homogenates so that comparison to whole fish tissue can only be made subjectively. Analysis of their data, in light of the kinetics of food oxidation, show what would be expected. For example, different metals had different catalytic activity, and amino acids had either pro or antioxidant ability, depending on the pH of the homogenate. Phenolic antioxidants significantly reduced rancidity; the more unsaturated the fat content of the fish the more rapid production of TBA.

The flesh lipids of cod and most lean fish are chiefly phosphatidyl ethanolamine (PE) and phosphatidyl choline (PC) which contain highly unsaturated fatty acids. PE is probably the primary cause of initial changes leading to rancidity in lean fish. C_{20}:5, C_{22}:4, and C_{22}:6 fatty acids of PE are particularly labile, and when oxidized in the presence of protein, give rise to both soluble and insoluble material, the latter being polymerized protein together with bound and/or trapped lipid. In the case of cod, jelly-like flakes of suspended material can accumulate to a stiff gummy mass. It is postulated that this close relation of phospholipids and the cell allows for direct attack of the lipid peroxy free radicals on remaining cell constituents.[263]

Oxidized lipids are more effective than unoxidized in aggregation of myosin.[276] It takes only 10 to 15 mg fatty acid peroxide free radical

TABLE 10

Rates of Oxidation of Fish Tissue[272]
μl O_2/gram fish/day

Fish	Fresh tissue 0°C		Freeze dried 30°C	
	Light meat	Dark Meat	Light Meat	Dark Meat
Chum Salmon	0	408	48	816
Pink Salmon	0	300	1056	8880
Tuna	0	310	-	672
Sheepshead	9.6	36	-	-
Lake Chub	14.4	-	-	-
Pilchard	168	-	7200	-
Rockfish	24	-	-	-
Cod	12	-	-	-
Mackerel	192	-	7200	-
Sole	-	-	72	-
Halibut	-	-	48	-
Cod	-	-	19	-

to denature all the myosin (MW 500,000) of 100 g cod muscle. This is approximately equal to a peroxide value of 60 which is in the range of initial unacceptability.

Lipid oxidation proceeds much more rapidly in the fatty species (Table 10); the lipids being in greater concentration and less dispersed through the tissue. The rate of oxidation in fish tissue is about 5 to 10 times slower than of the extracted oil.[277] This is reasonable since the proteins and other interfering substances should decrease the rate although in some species, such as the salmon, very little difference was observed.

Frozen Fish

Rancidity development in frozen fish depends to a large extent on storage temperature, and, of course, species of fish.[275,278-280] Lipid oxidation and phospholipid hydrolysis proceed rapidly at temperatures between 0°C and -12°C to -18°C, with maximum rates at about -4°C. Below -18°C the rate of oxidation decreases rapidly.

In freezing, much of the free water in the tissue is effectively "removed" by crystallization, thereby concentrating catalytic salts and reactants. In a sense, frozen fish will behave as if in the intermediate moisture range where oxidation is accelerated.

Oxidative rancidity in frozen cod has been studied extensively.[268,281,282] No rancidity developed when fillets were stored at -25°C for 132 days or at -18°C for 100 days. TBA values began to increase, though, when fillets were stored at -12°C. Rancidity did develop when fillets became surface dehydrated due to poor packaging and storage at -9 to -11°C. TBA values were still low, but odors described as "old dried salt fish" and "rancid fish oil" were observed. Iced trout has about only 1 to 1½ weeks storage life due to rapid rancidity development.[283,284] This acceleration in oxidation just below the freezing point is a complex phenomenon related to the increasing concentration of solutes in the unfrozen water and the decrease in the rate constant with temperature.

The major problem in frozen storage is toughening, which is associated with the thaw drip that occurs. The basis for this reaction has not been established because of the complexity of the reaction. One proposal has been based on the effect of the hydrolyzed lipid (free fatty acids) that appears during frozen storage. The research group at Gloucester showed that free fatty acids can accelerate myosin aggregation in model systems.[285,288-290] Jones,[274,286] Ackman,[266] and Dyer[287] have recently reviewed this area. It was shown that neutral lipid may protect the proteins from this effect. According to Olley et. al.[291] protein denaturation occurs rapidly in both lean and fatty fish during frozen storage. For the lean no neutral lipids are present to counteract the free fatty acids, for the fatty fish the lipids are not available. The slightly fatty fish are the most stable since enough lipid is available. This whole question was hotly debated at a NATO sponsored conference in Aberdeen, Scotland in 1969 but no real conclusions were drawn. Connell[293] and Dyer[294] recently reviewed the area of protein denaturation in frozen fish.

Another deteriorative problem that seems to be related to lipid oxidation in frozen fish is the "rusting" that occurs. This occurs on the surface of fatty ocean species with the development of yellow-brown discolorations. In rosefish at -12°C, this occurs in about 10 weeks.[278] It has been proposed that this is due to nonenzymatic browning from natural carbonyls reacting with the amines that were produced by surface bacterial decomposition. Furthermore, since lipid oxidation products are carbonyl compounds, they also can react in the same pathway.[292] By applying a surface glaze of water to the fish, lipid oxidation and rusting are prevented. Antioxidant dips have little effectiveness in increasing the shelf life of frozen fish, especially for the very fatty species.[287] The reported storage life of frozen fish is much less than that of beef or chicken.[294] For example, at -12°C lean fish have a storage life of one month, fatty fish less than two weeks, whereas beef is acceptable for about four months. Fatty fish must be kept below -30°C for four months stability.

Dehydrated Fish

For some fish it seems that oxidation of fats takes place more rapidly in the dehydrated state than in the fresh state (Table 10). However, the results for the dehydrated fish are at 30°C compared to 0°C for the fresh tissue. Assuming a $Q_{10°C}$ of about 2 to 3, the fresh tissue values at 30°C would be about 10 x larger. In this case we find fresh chum salmon oxidizing faster than the freeze-dried, as found with beef, turkey, and chicken. Fresh mackerel, on the other hand, oxidizes slower than dried tissue. This is a very

complicated situation and more research is needed to understand the differences due to the presence and absence of water.

Some fish products, such as dehydrated fish meal, oxidize so rapidly that spontaneous combustion may result.[274] Fish meal usually consists of the whole fish ground up and dried so that there is very intimate contact of the lipid with the minerals in the bones, scales, and blood. A very recent study of Chilean fish meal showed the reason for the very rapid oxidation rate was that the 6% moisture content is in the accelerating intermediate moisture range of the isotherm (A_W = 0.55).[295] If the product is dried further it becomes more stable. This study also showed that because oxidation is so rapid, (250 to 700 $\mu l O_2$/g/day) diffusion control by the particle size is significant. Maximum rate kinetics were appropriately applied in this study. The low peroxide values found, as compared to the oxygen reacted, are consistant with the close proximity of the lipid and protein in the meal. Using higher temperatures in drying also produce a more stable meal, probably due to increased browning products which have antioxidant properties.[156] Antioxidants also work very well because they can be blended into the meal easily.

With freeze-dried salmon at 37°C, the oxidation pattern follows the maximum rate kinetics scheme (Figure 12[10]). Oxygen absorption was about 90 μl O_2/g/day much lower than reported in Table 10 by another group. This could be due to all kinds of factors such as sex, age, time of catch, etc. It is interesting to note that the peroxide levels reached a reasonably stable maximum value very quickly and kept at that level for over 30 days, supporting the contention of maximum rate kinetics. Even under these conditions, however, the expected effect of water acting to inhibit oxidation took place as would be predicted. The monomolecular rate constants K_m reported were in the same range as for linoleate model systems when calculated on a lipid basis verifying the basic initiation mechanism proposed for foods. Degradation of the fat soluble carotenoids also followed the same pattern. With cod, storage at 37°C even in nitrogen (probably < 2% oxygen), rancidity develops rapidly becoming unacceptable in one month even though being a low fat fish.[296] Below 65°F little deterioration occurred. The samples showed quite a bit of surface rusting as in the frozen storage.

In conclusion, fish are much more susceptible to oxidation and the effect of water is unclear. Further research is needed to understand the reaction kinetics.

Milk and Dairy Products

Fluid Milk

Milk and its derivative dairy products are probably the most studied systems in terms of lipid oxidation. Badings[298] has made an excellent review of the flavor and odor compounds responsible for the rancid odor in liquid milk and isolated butterfat. Milk fat is almost entirely triglycerides (~ 99%) which are originally in a fat globule surrounded by a membrane composed of phospholipids and proteins. The triglycerides have a significant amount of saturated fatty acids of short chain length, such as butyric acid (C_4). The phospholipids also are low in unsaturated acids (only 6% linoleate).[299,300] Milk also contains between 100 to 250 μg iron/g milk and 20 to 40 μg copper/kg which, since the fat content is about 3 to 4%, gives trace metals in the ppm range, sufficient to be active as catalysts. The question of the integrity of the globule in protecting the fat from oxidation is very intriguing and has led to much speculation. Brunner[301] has summarized the various theories of the globule structure and from this it is impossible to conclude what exact effect may be attributed to the structure itself on oxidation rate.

Exposure of liquid milk to sunlight produces an off flavor, supposedly due to oxidation of lipids and possibly proteins.[302] Both sulfhydrl and carbonyl compounds have been implicated as well as identified.[303,304] It is also felt that copper is responsible for production of an oxidized flavor in milk resembling a cardboardy odor. The milk industry has eliminated the use of copper vessels and lines, but the natural copper is still present in high enough amounts. Emulsion studies of milk fat have shown that the rate of the copper-induced rancidity was influenced by the amounts of sugars, proteins, and salts present. These additives strongly influenced the catalytic action of added ascorbate.[307,312] Haase and Dunkley[308] have recently reviewed the area of copper-induced oxidation flavors in milk. EDTA can reduce this copper problem; however, it cannot be added to milk because of the standard of identity.[305] No studies have been made of the direct oxidation rate of liquid milk. With homogenization and pasteurization, it should be expected that

rancidity could be a problem in milk stored for over one week if its initial microbial count is low. Otherwise, bacterial growth usually is responsible for the short storage life of milk.

All milks do not have the same susceptibility to oxidation. The stability depends on many factors, including the type of feed which seems to be most important. Day has reviewed the area of off flavor development in milk and has shown that the fluid product becomes rancid at peroxide values much less than one. This is because milk has a very bland flavor and since the oxidative products have very low flavor thresholds, they are detected at low levels (Table 3). Kinsella[309,310] has updated this review recently. It is interesting to point out that the types and amounts of off flavors produced vary with the physical state of the product. Thus, fluid milk develops a cardboardy flavor indicative of alk-2-enals, whereas dry milk gets an oily or tallowy odor of hexanal. These different end products are formed even though the same lipid is oxidizing. Wilkinson[306] has proposed pathways to explain these differences and has further expanded this to show how oct-1-ene-3-one could form under different conditions of water content, oxygen tension, and lipid type.[311]

Milk Fat

Much work has been carried out on the oxidation of milk fat itself. It has been shown that various light sources promote oxidation of milk fat, not just in the ultraviolet region.[313] Hammond and Hill[314] have shown that oct-1-ene-3-one comes from linoleate oxidation as did Wilkinson. They describe this compound as responsible for the metallic flavor of milk. It is the best kinetic study available.

Hamm et al.[315] oxidized milk fat over a temperature range from -27°C to +50°C. Oxidation was followed by peroxide value and TBA as well as for type of off odor in order to determine the reliability of an accelerated storage test. It was found that both the oxidized metallic and tallowy flavors were produced more rapidly at -27°C compared to -10°C. Above -10°C, however, the rate of production increased with temperature. Peroxide increase and TBA increase did not show this negative temperature effect. They plotted the peroxide and TBA data on a semilog plot. Although there was scatter, the lines fit the data well as might be expected for oxidation taking place in the presence of natural antioxidants, following Equation 29. The reason that was given for using a semilog plot was that initiation occurs by a bimolecular mechanism. This is doubtful unless they carried the oxidation very far which is not the case (maximum PV reported was < 1). The logarithmic plot would follow both cases but the natural antioxidant case would seem to be more acceptable for low extents of oxidation.

From their data they found an activation energy of 10.3 kcal/mole for the peroxide formation and 13.9 for TBA value. This seems reasonable in light of the kinetics discussed above. Also interesting, is that the temperature covers the range of both liquid and solid fat but the absolute rates all fall on the straight line on an Arhennius plot. The major mistake they have drawn in their conclusions is that since the activation energies differ, different fatty acids must be involved in the TBA and PV production. Most likely it is the same fatty acid, but since TBA measures a product further down the reaction sequence from a peroxide, one must add the extra activation energy of those steps. They also found that although added copper changed both the induction time value and slope of the oxidation plot, the activation energies did not change. This means that since they were already starting out with catalyzed oxidation, increased metals thus would show the observed effect. They explain it on the basis that copper increases the concentration of RH by oxidizing other types of fatty acids. This seems to be a fallacious reason. Finally, they tried to determine activation energies for the off flavor production. It was found that the plots were much more scattered and had an activation energy of about 8, less than that of either TBA or peroxide numbers. No reasonable explanation can be given for this, since it should be either the same or higher. They state that the values were not statistically different from the peroxide value. This seems reasonable, since the methods used for flavor analysis by a panel could influence the results creating scatter.

Gilchrist et al.[316] have shown with butterfat that storage at temperatures of 21°C for three weeks still gave an acceptable butter if microbial growth were prevented. These data fit the low peroxide value found over the same period by Hamm et al.[315] According to that, data storage at -10°C would keep the butter acceptable for over 5 months. However, when the butter is exposed to light, surface oxidation occurs rapidly causing off

odors. McPowell[316] has also pointed out the necessity of keeping the copper level very low to insure stability in storage.

Dried Milk

The major work of the influence of oxygen level, moisture content and temperature of various types of dried milk products has been done at the Dairy Products Lab of the Eastern Utilization Research and Development Lab.[318-325] Dehydrated milk products are a major business as indicated recently in a business review of food processing.[326] Nonfat dry milk (~ 1% fat) was used at the rate of 1.02 billion lbs. in 1969 and dried whole milk at 50.2 million lbs.

As would be expected, dried whole milk (~ 25-30% fat) is a much more unstable product than nonfat milk. Levels of oxygen of less than 1% are needed to give sufficient shelf life at room temperature. In air the first faint rancid odor is noticed in about one week, which is similar in rate to the development of off flavors in fluid milk. If the oxygen level is at 1%, storage life of foam dried milk increases to 3 weeks, and is 4 to 5 months at 0.1% oxygen. Commercial packaging by vacuum and nitrogen flushing usually cannot get the level below 1%, therefore, shelf life is very short. Spray dried whole milk has a slightly longer shelf life, possibly due to production of anti-oxidants during drying at the higher temperatures. The results with oxygen level follow the predicted pattern very well based on the lipid oxidation kinetics previously discussed.

The work of these laboratories also pointed out the complicated influences of oxygen level and moisture content. As expected, the milk powder at 4% moisture was significantly better than at either lower levels (2 to 3%) or at 5% moisture. The 4% moisture is near the calculated monolayer level (3.96%), below this oxidative deterioration occurs, above this nonenzymatic browning is accelerated (Figure 14). Storage temperature also showed the expected pattern. Stability was best at 34°F; above this flavor scores dropped rapidly. At 0°F the product was also less stable, possibly because the fat is in the solid state and propagation steps of oxidation are favored as indicated previously. The authors, however, did not consider this possibility. Moreover, at low moisture levels reduction of oxygen level is not as effective. The oxidation becomes more like that of a pure lipid where very low levels of oxygen are necessary to reduce the rate of oxidation. The overall stability of dried whole milk seems to be much less than that of the dehydrated meat products discussed previously, probably due to the closer contact of metals and oxidizable lipid. However, no oxygen absorption data have been reported. Based on peroxide values the oxygen absorption is slower, about 4 to 5 μ 10_2/gram/day. The initial oxidation level is much less starting at peroxide values of less than 0.02 (< 0.0002% oxidized), but since the product is bland, rancidity is detected more easily.

Nonfat dried milk presents no problem oxidatively because the susceptible lipids are removed during processing. It has a storage life of over one year in air at room temperature.

CONCLUSIONS

Lipid oxidation is a very complex reaction of unsaturated lipids with oxygen. In foods it is further complicated by the presence of metals, amino acids, proteins, and by how and where the lipid is compartmented. This review had the intention of reducing the difficult kinetics of oxidation to a simpler form. The usefulness of this will be in the application of these kinetics to analysis of oxidative rancidity of foods. Much previous work has not considered the reaction kinetics, making the usefulness of the data minimal. It is hoped that future researchers will consider the kinetics problem, and apply it to their studies of food stability. Only in this way can one hope to understand the reaction complexity in food and to apply the data to predict storage life.

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