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AN ASSESSMENT OF LIPID OXIDATION IN FOODS

(Technical Report)

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An assessment of lipid oxidation in foods (Technical Report)

ABSTRACT

Lipid oxidation has been long recognized as a major problem in the storage of fatty foods. Oxidative changes can result in repugnant flavors, in destruction of valuable nutrients and even in generation of toxic compounds. This reaction occurs by several molecular mechanisms which all channel to generation of oxygen-rich precursors of reactive, chain-propagating, free radicals. The complexity of the chemistry involved defies the possibility of one, universal, analytical test for unconditional evaluation of the oxidation deterioration. However, the better understanding of lipid oxidation achieved in the recent years should assist in answering the questions of importance in food systems.

INTRODUCTION

The reaction of molecular oxygen with organic molecules has for long been a process of considerable interest. Although a wide variety of organic molecules are susceptible to chemical attack by oxygen, a great deal of attention has recently been focused on lipids because of the remarkable implications of their oxidative damage. At the biological level, the oxidation of lipids means damage to membranes, hormones and vitamins, which are vital components for the normal cell activity (1). At the nutritional level, the oxidation of fatty constituents is the major chemical factor in the loss of food wholesomeness by deterioration of flavor and aroma, as well as in decay of nutritional and food safety qualities (2). Recently, biological and nutritional aspects have merged; diets based on food containing peroxidized lipids have been related to far-reaching effects such as carcinogenesis, premature aging and other diseases (3).

Oxidation of lipids can occur in foods containing substantial amounts of fat, like milk and meat products, oils, nuts and also those that contain only minor amounts of lipids, such as vegetable products. Practically all quality attributes of food can be affected by this process. Thus, aroma changes result from new volatile odorous compounds formed, flavor modifications are caused by hydroxy acids, the color darkens as the result of condensation reaction between oxidation products and proteins, and finally, a new texture might be attributed to the oxidative induction of protein crosslinks. Not unexpectedly, the nutritive value and safety of food are impaired.

This study is not intended as a comprehensive review of the literature but as an assessment of directional ideas relevant to food chemistry, in what is often a complex multidisciplinary field.

CHEMICAL ASPECTS

Classical studies established the mechanism of autoxidation of lipids as a free radical chain reaction which involves the three stages of initiation, propagation and termination (4) (Scheme 1).

Scheme 1. The autoxidation reaction

Initiation	$RH + ?$	$\rightarrow R\bullet$	(1)
	$R'\text{-}CH{=}CH\text{-}R'' + O_2 \rightarrow ?$	$\rightarrow ROOH$	(2)
Propagation	$R\bullet + O_2$	$\rightarrow ROO\bullet$	(3)
	$ROO\bullet + RH$	$\rightarrow ROOH + R\bullet$	(4)
	$ROOH$	$\rightarrow RO\bullet + OH\bullet$	(5)
Termination	$R\bullet + R\bullet$	$\rightarrow$ Nonradical products	(6)
	$2ROO\bullet$	$\rightarrow R_1\text{-}CO\text{-}R_2 + R_1\text{-}CHOH\text{-}R_2 + O_2$	(7)
	Other free radical rearrangements		

The initiation step is the most intriguing aspect of this chemical process. The spontaneous abstraction of a hydrogen atom from an organic material by molecular oxygen (equation 1) is an endothermic reaction which demands a large activation energy and although it might occur to a certain extent, it is probably too slow to be of practical importance. Alternatively, the direct addition of an oxygen molecule to a double bond to generate hydroperoxide compounds (equation 2) is prevented by the spin conservation rule due to the triplet state character of the ground state oxygen. Therefore, either the organic molecule or the oxygen should be activated before reaction.

The generation of the primary radicals is facilitated by accidental or intentional presence of oxidation initiators such as transition metals, oxidants, various homolysis-prone substances or enzymes. Transition ions, like iron and copper, have a range of accessible oxidation states which assist in catalysis of electron transfer reactions. The redox potential is variable as a function of the ligand. Stable paramagnetic states resulting from the presence of unpaired electrons are common for transition metals and facilitate their reactions with other organic radicals and biradical molecular oxygen. Transition metals may interact directly with triplet oxygen to generate superoxide radical anion or may be involved indirectly in the generation of oxygen species by oxidizing enzyme co-factors like flavins which in turn can activate oxygen *via* the transient semiquinone radical.

In the absence of xenobiotic compounds, the most important sources of primary free radicals *in vivo* are processes that are controlled by endogenous enzymes and which generate highly reactive free radicals. Microsomal lipid peroxidation mediated by flavoenzyme/ NADPH/ cytochrome-P450 reductase, was demonstrated in foods, particularly in muscle foods. Lipoxygenases, the non-heme enzymes that contain iron in the active site, can catalyze 1,4 pentadiene fatty acids to hydroperoxides. Xanthine oxidase, a metallo flavoprotein, can oxidize several substrates with concomitant reduction of oxygen to superoxide radical. Cyclooxygenase is an enzyme which oxidizes arachidonic acid to prostaglandins and its activity may elevate the "peroxide tone" in many tissues including muscle foods after slaughtering (5). These enzymatic processes often involve superoxide radical anion O_2^- and its disproportionation product, H_2O_2. Because of its lack of reactivity, O_2^- cannot be directly responsible for the initiation of the lipid oxidation chain. The concept of oxygen activation through O_2^- has been consequently revised and the possibility of O_2^- conversion to some other oxidizing species, such as hydroxyl radical, as the result of an O_2^- - driven Fenton reaction (Scheme 2), has been suggested. The transition ion, M^{n+}, can well be provided by heme proteins.

Scheme 2. O_2^--driven Fenton reaction

$$2O_2^- + 2H_2O \rightarrow H_2O_2 + 2OH^- + O_2$$
$$M^{n+} + O_2^- \rightarrow M^{(n-1)+} + O_2$$
$$M^{(n-1)+} + H_2O_2 \rightarrow M^{n+} + OH\bullet + OH^-$$

Another possibility of activation of ground state oxygen is by electron excitation. The oxygen activated to the singlet excited state reacts much more readily with some unsaturated lipids than ground state oxygen; for instance, the reaction with methyl linoleate is at least 1500 times faster. In foods, a possible source for singlet oxygen is the photosensitization by dyes. The food pigments, natural and artificial, are likely to serve as promoters of such photochemical reactions due to their light absorbing capabilities and indeed, chlorophylls, riboflavin, FD&C red No. 3, have shown extensive photosensitive ability (6). The preference of the market for transparent packaging (and colorful food) creates the most suitable conditions for exposure of foods to light. Other hypotheses suggest that generation of singlet oxygen during lipid peroxidation might be attributed to breakdown reactions of lipid hydroperoxy free radicals. These possibilities are based (a) on Russell's rearrangement and assumes the self-reaction of lipid peroxy radicals (ROO•) with the formation of an acyclic intermediate which may decompose into either a triplet state carbonyl compound or else yield singlet molecular oxygen , (b) the formation of a tetroxide followed by decomposition and (c) reversal of a hydroperoxy radical to lipid free radical and singlet oxygen (Scheme 3).

Scheme 3. Potential pathways for dark generation of singlet oxygen

$$2R'R''HCOO\bullet \rightarrow R'\text{-}CO\text{-}R'' + R'\text{-}CHOH\text{-}R'' + {}^1O_2 \quad (a)$$
$$2ROO\bullet \rightarrow ROOOOR \rightarrow 2RO\bullet + {}^1O_2 \quad (b)$$
$$ROO\bullet \rightarrow R\bullet + {}^1O_2 \quad (c)$$

According to this hypothesis, singlet oxygen is actually a product of a side-reaction channeled through peroxy radical decay and consequently, singlet oxygen might be regarded as a consequence of lipid peroxidation rather than an initiator of the process. The possible generation of singlet oxygen from peroxy radicals has been a subject of dispute (7).

The food, as a whole, is a particularly complex chemical matrix and it is clear that no one mechanism can be held exclusively responsible for the initiation of the lipid peroxidation. While in the raw foods the enzymic oxidation plays a most significant role, in the processed foods, the chemical initiation is probably determinant. The understanding of the activation process of the oxygen and the reactivity of the species formed, gives the necessary insight into feasibility of lipid oxidation reaction and possibility of prevention. Since every form of "active" oxygen has its specific inhibitor, the detailed understanding of a particular oxidation process is needed for tailoring a suitable antioxidant.

Once a free radical is generated, the chain reaction of oxidation is initiated, new free radicals, carbon- and oxygen-centered are formed and the process is easily propagated. The net chemical result of lipid oxidation is very complex (8). Multiple initial products result from one starting material and many more are generated by the decomposition of instable hydroperoxides. This decomposition proceeds by homolytic cleavage of a peroxy bond to form alkoxy radicals. These radicals undergo carbon-carbon cleavage to form breakdown products including aldehydes, ketones, alcohols, hydrocarbons, esters, furans and lactones. Lipid hydroperoxides can react again with oxygen to form such secondary products as epoxyhydroperoxides, oxohydroperoxides, bihydroperoxides, cyclic peroxides and bicyclic endoperoxides. These secondary products can in turn decompose like monohydroperoxides to form volatile breakdown products. Alternatively, the hydroperoxides can condense into dimers and polymers (9,10). A large variety of analytical tools has been applied to the study of lipid oxidation and the characterization of the products, primarily in model systems: liquid-, gas-, thin layer- chromatography, countercurrent distribution, gas chromatography-mass spectroscopy, high pressure liquid chromatography, ^{13}C nuclear magnetic resonance (11).

The oxidation products of lipids can also interact with proteins. At the biological level, the coexistence of lipid and protein moieties in one complex form is of a crucial importance for the structure of cell membrane. In food systems, major functions of these lipid - protein "clusters" include stabilization of dispersions against coalescence and participation in the formation of texture. Consequently, it is expected that changes in lipid - protein interaction, for instance due to oxidation of the lipid moiety, will affect the texture of liquid or solid foods. Damage to proteins initiated by lipid peroxidation has been observed in model laboratory systems and strongly suggests that similar interaction between peroxidizing lipids and proteins may also occur in the matrices of foods (12). This interaction may proceed along two different chemical avenues. In one potential process, transient intermediates in lipid oxidation such as free radicals and hydroperoxides react with proteins. This reaction yields protein-centered free radicals and subsequent structural alterations. Alternatively, proteins react with stable lipid oxidation products such as aldehydes, ketones, epoxides or oximes to yield new products or crosslinks between protein chains. Particularly, bifunctional secondary products of lipid oxidation such as malonaldehyde are powerful crosslinking agents which react with amino groups of enzymes and proteins (13). It is expected that similar chemical interactions might affect any other food components such as vitamins and carbohydrates.

Fundamental differences exist between the oxidation of lipids at high temperatures and the oxidation at ambient temperature. The thermal activation can drastically change the further reaction pathways of the initial oxidation products.

Obviously, the course of oxidation is also dependent on the presence of antioxidants present in food. To date, the most common food antioxidants are synthetic additives which trap and stabilize the free radicals responsible for propagation and branching reactions and consequently act as chain breakers. Among them, phenol-type compounds such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are dominant.

An attractive alternative is the use of tocopherols. These natural antioxidants exhibit a dual type of activity: free radical scavengers and chain breakers, as well as physical quenchers of singlet oxygen (14).

Besides these antioxidants, there are also compounds which inhibit the initiation step. The most important initiation suppresors are metal scavengers which bind the metal ions catalyzing chain initiation. This group includes citric, phosphoric and ascorbic acids.

A large variety of natural compounds have been shown to inhibit oxidation under very specific experimental conditions: amino acids, proteins, enzymes, phospholipids, plant and spices components (flavonoids, carotene, phenols). Antioxidative effects were also obtained by chemical changes caused during food processing. Thus, Maillard reaction products, fermentation products, protein hydrolytes, food smoke, have been claimed to exhibit antioxidative effects. The inhibitory effect of these compounds is dependent on many experimental factors, the active principle in some of these systems has not been characterized and the mechanism is not entirely clarified. Consequently, their practical significance is limited.

It is obvious from this brief presentation of the chemistry of oxidation that the chemical composition and the physical state of a certain food determine the route, rate and final effect of lipid deterioration.

ASSESSMENT OF LIPID OXIDATION

Innumerable factors influence the relative amounts of reaction products formed by lipid oxidation and a wide range of tests, from simple organoleptic evaluation to physical and chemical methods of various complexities, have been suggested (15). Table I summarizes a few chemical assays of lipid peroxidation.

In spite of the multitude of assays, an universal method which correlates well with the extent of food deterioration throughout the entire course of autoxidation, is not available. The present methods give information about particular stages of oxidation process and some are more applicable to certain lipid systems than the others. This situation should not be unexpected in view of the chemical diversity of the food matrices and of the oxidation pathways.

When the process of oxidation is followed comparatively in the course of time, the loss of lipid substrate or the amount of oxygen uptake can serve as general, though nonspecific and usually not sensitive enough, indexes for peroxidation of lipids.

Since the primary products of lipid oxidation are hydroperoxides, it is reasonable to determine their concentration as a measure of oxidation. The "peroxide value" test reflects the total concentration of peroxides and hydroperoxides present at a certain time. However, this approach is restricted by the chemical instability of these compounds; after their concentration reaches a maximum level it decays as a function of temperature, the presence of other food components, etc.

An alternative approach to the determination of the extent of oxidation is the measurement of products of hydroperoxide degradation. In contradistinction to the peroxide determination, such an assay is not limited to the early stages of oxidation and may reflect the formation of products, like carbonyl compounds, which actually contribute to the rancid and other objectable organoleptic flavors. The application of methods based on this approach requires a detailed knowledge of the chemistry involved, stability of the compounds assayed, etc. Among these methods, "thiobarbituric acid test (TBA)" is one of the most common, in spite of criticisms of its reproducibility and even reliability. The test is based on the color product resulting from the condensation of TBA with malonaldehyde which is presumably generated in the oxidized fats. However, a large body of evidence suggests that other food components can react with TBA to generate the same chromophore and even the formation of malonaldehyde is dependent on the composition of the initial lipid.

Table I. Compendium of assays for lipid oxidation

Monitored reaction effect	Method of assay
Loss of lipid substrate	Gas chromatography[a]
Oxygen uptake during oxidation	Oxygen uptake[a]
Formation of peroxides, hydroperoxides	Iodometry[b], enzymatically[c], chemiluminescence[d]
Formation of malonaldehyde	Direct determination by UV absorption (λmax=245 nm)[e] or HPLC[f], derivatization with thiobarbituric acid (absorption at λmax=532 nm or fluorescence at λem=553 nm)[e]. Fluorescence of Schiff base (λem=455 nm)[g].
Formation of conjugated dienes	Increase in OD at λ=233 nm[h]
Formation of carbonyl compounds	Direct determination by GS-MS, HPLC, derivatization with 2,4-dinitrophenyl-hydrazine, etc[i].
Formation of free fatty acids	Titration, electric conductivity[j].

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The assays of oxiranes, conjugated dienes, trienes, aldehydes and fluorescent compounds are some of many other assays based on quantitation of suspected end products of lipid oxidation.

Finally, the oxidative decay of lipid components in food is reflected, most significantly, in objectionable flavors and taste. Sensory evaluations of foods by testing panels, with the results statistically analyzed, may serve as an assay for evaluation of oxidation extent.

CONCLUSIONS

The great deal of knowledge accumulated on the general chemistry of oxidation of lipids suggests that further efforts should be directed to a detailed understanding of the effects of this process in specific foods. Potential targets for future research are:

- Clarification of the initiation step of oxidation in specific foods and ways for prevention or inhibition.
- Development and standardization of one or a set of analytical assays for evaluating the extent of lipid oxidation in specific foods and implicitly the extent of oxidative decay. Evaluation of IUPAC standard methods for oils and fats on extracts of lipids from foods.
- Development of "oxidation index" for specific foods which would be useful in assessing the potential for lipid oxidation and implicitly the food stability. The lag phase preceding the exponential phase of oxidation could be a constituent of a such index.
- Recommendations for isolation of oxidized lipids in individual foods under conditions that avoid decomposition of the unstable products and formation of new ones. Study of methods for lipid extraction.
- Investigation of the oxidation products from lipids other than fatty acids (e.g. cholesterol).
- Investigation of the synergistic effects of peroxidizing lipids and adjacent food constituents. Determination of the decrease in antioxidant levels (e.g. tocopherol, ascorbate) or of increases in the oxidized products from antioxidants (e.g. tocopherol quinone or the ascorbyl radical). Detection of oxidized products from proteins (e.g. methionine S-oxide from methionine).
- Establishment of acceptable limits of oxidation based on criteria of wholesomeness, nutritional and safety values. Some guidance is provided by Codex Alimentarius that accepts levels of 10 meq/kg of peroxides in fats and oils for edible purpose.

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