CHEMICAL FACTORS AFFECTING DEGRADATION PROCESSES OF VEGETABLE OILS DURING FRYING

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A Dissertation submitted to the

Graduate School-New Brunswick

Rutgers, The State University of New Jersey

in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

Graduate Program in Food Science

written under the direction of

Professor Karen M. Schaich

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New Brunswick, New Jersey

October, 2013

ABSTRACT OF THE DISSERTATION

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Food oils degrade rapidly when exposed to high temperatures in frying, but the mechanisms responsible have never been fully elucidated. Thermal degradation is usually described as a combination of hydrolysis and accelerated autoxidation. Thermal scissions of lipid chains have also been identified but remain controversial and largely ignored. This research sought to elucidate the reactions underlying thermal degradation, reconcile roles of thermal scission and autoxidation, and determine where catalytic factors intervene in degradation at frying temperatures.

Thermal degradation processes were investigated in high oleic sunflower oil:corn oil blends (60:40 w/w) heated in an Oxipres^{TM} oxygen bomb at 180°C for three hours under 2 bars (O_2, air, or O_2/N_2 blends) to determine rates and amounts of oxygen consumed during heating. Degradation products (conjugated dienes, peroxide values, aldehydes, free fatty acids) were measured in samples removed at various time intervals. In a parallel study, volatiles released during heating were trapped and analyzed by GC-MS. Effects of oil state and pre-oxidation were evaluated using stripped, fresh, and steady-state frying versions of the oil blend. Effects of oxygen, metals, water,

phospholipids and free fatty acids were tested individually and in combinations to reveal synergistic catalysis of degradation.

Results supported thermal scission of lipid acyl chains as the dominant process controlling degradation at high temperatures, generating radicals that are the precursors for a broad range of downstream products. Terminal peroxyl radicals formed by addition of oxygen to thermal scission radicals abstract hydrogens from lipid chains to initiate autoxidation chains as a secondary process. Oxygen and water were the major forces driving secondary reactions. Traditional catalytic factors known to induce lipid oxidation had relatively minor effects alone but in combination with water triggered rapid consumption of O_2. Except for oxygen, catalytic factors affected secondary oxidation processes and product distributions rather than initiating oxidation. Mismatch between O_2 consumption and levels of oxidation products demonstrated presence of reaction pathways that consumed oxygen but led to products other than those measured.

Data was integrated to develop a new reaction scheme for thermal degradation of oils. Application of this new information can be used to improve stabilization of frying oils.

ACKNOWLEDGEMENTS

I would like to express my greatest gratitude and appreciation to my advisor Dr. Karen M. Schaich. She provided me with support and encouragement throughout my graduate study. She was always there for me when I ran into difficulties and steered me in the proper direction. I feel very lucky and grateful to have an advisor from who I learned not only an incredible amount of knowledge, but also the wisdom of how to think and solve problems.

To my dissertation committee -- Dr. Michael M. Blumenthal, Dr. Chi-Tang Ho and Dr. Chaim Frenkel -- I give my sincere appreciation and thanks for taking time from their very busy schedules to review my thesis proposal and dissertation, and for giving me insightful and helpful advice about my research.

I thank all my labmates: Jia Xie, Karen Chang, Wan Zunair Wan Ibadullah, Yuan Dong, Elah Steltzer, Ilona Repko, Lynn Yao, Brandon Bogusz, Teng Peng, Anna Puganen, Huidong Huang, Tony Liao, Emily Nering for their support, help and most of all, their friendship. I have treasured every minute I spent with them.

Finally, I would like to express my love and appreciation to my parents. Even though they live the farthest apart from me, they are the people who care about me the most. I would not have achieved anything without them.

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1. INTRODUCTION

Potato chips were invented by George Crum, an American chef, when he became so annoyed by one customer's incessant complaint about the thickness of his fried potatoes, that he sliced the potatoes very thin and fried them till crispy. To everyone's surprise the new food was well liked and became a famous item on the menu [1]. Today, potato chips have become one of the most popular snacks enjoyed by people around the world. Indeed, the unique flavor and texture of all fried foods, as well as the simplicity of preparation, have helped frying gain its popularity as a cooking method.

Foods adsorb oil during frying. Under high temperature, water in the food evaporates, leaving pores in the structure [2]. During the cooling stage, large amounts of oil enter these pores. Potato chips, due to their large surface-to-volume ratio, absorb up to 35-40% oil in the finished product [3]. It is well known that oil is susceptible to oxidation, so the quality of this adsorbed oil is very critical for the quality of the chips. Degradation of oil not only gives off unpleasant odor and taste, but also significantly reduces the nutritional quality of the food, and even worse, may promote or initiate carcinogenesis [4] or other toxic effects. Stabilization of oil quality is therefore critical for achieving desired shelf-life of fried foods, as well as for reducing potential health hazards [5].

During the 1960's and 70's, the food industry studied lipid oxidation extensively and thought they had learned everything necessary for limiting oxidation in foods. A major component of their approach was to increase more stable saturated fats and remove highly oxidizable polyunsaturated fatty acids, or to use hydrogenation to achieve stable saturated structures. However, in recent years, due to increasing consumer health

awareness of the potential health risks of saturated fats and trans fats on one hand, and the requirement of polyunsaturated fatty acids for health on the other hand, the food industry has been forced to reformulate products, abandoning traditional saturated fats for more healthy polyunsaturated and monounsaturated oils such as corn oil and sunflower oil. This reformulation, however, has posed great challenges for food manufacturers because unsaturated fatty acids are more susceptible to oxidation. Traditional approaches were no longer effective in preventing lipid oxidation and reformulated foods no longer could meet required shelf lives.

Reformulation with polyunsaturated oils has been a particular problem in frying because high temperatures lead to complex degradation of both saturated and unsaturated lipids. Overall degradation has been described traditionally as occurring by the following process (Figure 1):

1) free fatty acids are the first products in frying, derived from heat decomposition or hydrolysis of triacylglycerols;

2) oxidation of these free fatty acids generate desirable and off-flavors;

3) saponification of the fatty acids generates soaps and foaming;

4) free radical-mediated crosslinking, with and without oxygen, generates dimers and polymers that increase oil viscosity. Chemically, the mechanism has been attributed to normal autoxidation accelerated by Arrhenius kinetics at elevated temperatures [6, 7]. There are some conceptual problems with this explanation, however, since at typical frying temperatures of 150-180°C, the activation energy of many reactions is exceeded and reactions not occurring at lower temperatures become feasible. Therefore, different reactions or pathways beyond that of autoxidation may be expected to occur.

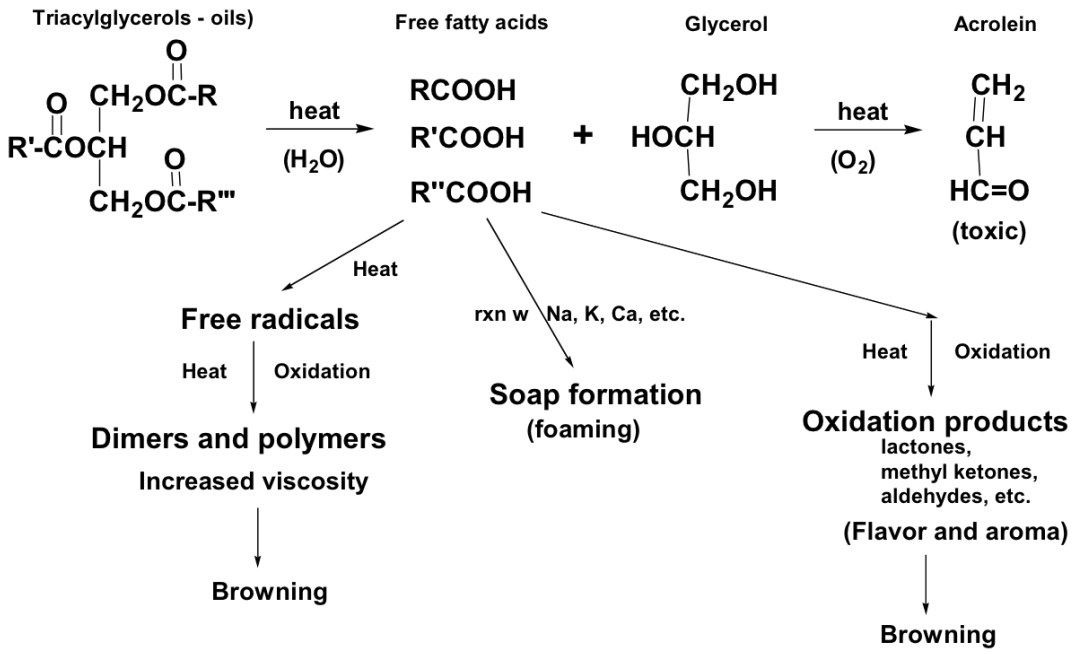


Figure 1. The overall process of frying oil degradation as most commonly portrayed. Adapted from schaich [8].

In 1969, Wassef Nawar [9] presented data supporting an alternate mechanism for thermal degradation of lipids – the thermal scission theory – in which high thermal energy cleaves bonds at all positions within the triacylglycerol structure, releasing free radicals that undergo reactions of their own as well as initiate autoxidation chains. This theory has not been widely incorporated into mechanistic explanations of frying degradation, and since then very little new understanding about the relationships between thermal scission and autoxidation processes in thermal degradation has been developed.

Difficulties in stabilizing polyunsaturated oils with traditional approaches based on the autoxidation theory have led us to re-examine the role that thermal scission plays in oil degradation at high temperatures. To better understand the relationship between the thermal scission and accelerated autoxidation mechanisms and possibly to differentiate their relative roles at different stages of frying, we heated oil under controlled conditions

in an oxygen bomb, with and without addition of components known to be oxidation catalysts, then followed oxygen consumption and concentrations of degradation products during three hours heating. Patterns of oxygen consumption and development of products provided interesting insights and new information about thermal degradation of oils.

Numerous research studies have been conducted to investigate factors affecting oil oxidation at room temperature or sometimes slightly elevated temperatures such as 40°C or 60°C. Fewer studies have dealt with influence of these factors at frying temperature and conclusions from these studies are controversial. In these, only effects of individual factors were studied and none examined combinations of conditions (more apropos to actual frying) to evaluate synergistic effects. To better understand the roles of frying conditions and oil components on degradation rate and pathways, we studied six categories of well-known factors (pre-oxidation, O_2, water, metal, fatty acids, phospholipids), evaluating them one by one and also in some two-way and three-way combinations. Results from these factors investigations may identify useful measures to improve oil stabilization.

Our group's frying project was composed of several sub-projects: 1) validation of the oxygen bomb for studying thermal degradation [10], 2) thermal desorption studies of volatiles released during thermal degradation of oils [11], and 3) the present study following effects of catalytic and protective factors on oxygen consumption and formation of several classes of oxidation products, and a parallel analysis of volatile products released from the oxygen bomb and collected on thermal desorption traps [12]. Data presented here focuses on non-volatile products, with connections to the volatiles study.

2. LITERATURE REVIEW

2.1 Frying

Frying is one of the oldest and simplest methods of food cooking, and it remains very popular. Frying involves putting food in contact with hot oil for various periods of time to withdraw the thermal energy and cook the food [13]. It is difficult to tell how far the technique goes back in time. The Old Testament from 600 BC distinguished between bread baked in the oven (Leviticus 2:4) and cooked in oil in a pan (Leviticus 2:7). In the first century AD, Roman authors described frying eggs. Then writers such as Cervantes and Chaucer in the Middle Ages described cooking in oil [14]. No matter where or when frying originated, it remains a cooking method widely used domestically, commercially and industrially [15]. Frying is fast, generates unique flavors and textures which cannot be created by other means, and can be accomplished with simple equipment [13].

The selection of frying oils, however, is not an easy task. An ideal oil meets all the challenges from operational performance to quality and stability to nutrition. First, it is critical that the physical and chemical properties of the oil match the frying application. Critical properties include melting point, heat content and heat capacity, and smoke point. In the case of industrial frying, frying oil stability is also important. Frying oil stability previously was achieved primarily through use of products that are more saturated, either naturally or by hydrogenation [16]. However, hydrogenation creates trans fats, which have (rightfully or wrongfully) been associated with increased risks of heart disease[17, 18] Saturated fatty acids have also been linked to heart disease and atherosclerosis [19, 20]. Today’s consumers are very health-conscious, and are concerned not only that foods are pathologically safe (will not contribute to long-term diseases) but also are

physiologically sound (have high nutritional quality to support health). To meet consumer expectations and demands, food industries around the world are reformulating for healthier diets, abandoning traditional saturated fats and hydrogenated oil for healthier monounsaturated and polyunsaturated oil [21, 22]. However, this reformulation put stability issues back onto the table [23].

2.2 Commercial frying versus industrial frying

The frying process used commercially, i.e. in catering restaurants or fast food operations, are different from the frying process used in the food industry. Commercial frying is usually batch frying where oil is heated to the desired temperature in a contained unit, then food is added in single batches at a time [24]. Usually, food is transferred to the fryer in a basket that is lowered manually or mechanically into the oil bath where the food is submerged and fried. The temperature and the amount of oil are maintained by electronic controls. Batch frying is suitable for small operations and preferred for specialty products.

In contrast, industrial frying that deals with large amounts of food uses continuous fryers. Here, raw food is continuously fed into one end of the fryer; propelled through on a submerged conveyor, conveyor pocket, or by convection; then removed cooked from the other end. In some designs, paddle wheels ensure uniform product distribution, regulate flow and submerge the product in hot oil [24]. Because large amounts of food are being fried continuously, industrial frying has shorter turnover rates than commercial frying. Oil turnover rate is defined as the number of hours before addition of make-up oil is required to maintain the amount of oil in the fryer [25]. Thus, for stabilization and

controlling oil quality in industrial frying, focus on early stages of frying is most critical. Moreover, fried products from commercial frying are usually consumed within a short time whereas industrial frying generates products that are marketed and stored, so require a reasonable shelf-life (at least several months). This provides a second reason why control over frying oil quality has become a serious concern for continuous frying in the food industry. This dissertation focused on industrial frying by using short time frying (3 hours).

2.3 Heat and mass transfer in frying

Frying is a process where mass transfer and heat transfer take place spontaneously. Heat transfer is achieved by convection within the oil and by conduction between the oil and food surface as well as within the food. The impact of heating in a frying process is determined by the final temperature and the rate of heating. Keeping the volume of oil large compared to food provides a huge heat reservoir to heat the food at a fast rate. In deep frying, where the food is surrounded by oil, heat is conducted uniformly to all dimensions of the food [26]. Water, which is important in mass transfer, also plays critical roles in heat transfer. The conversion from liquid water to steam carries off excessive heat from the oil, so that food surface will not burn or char. The heat is then conducted from the surface to the center. As a result, the interior of the food is cooked, which in most cases means the gelatinization of the starch [2].

Mass transfer involves migration of water and water soluble substances out of the food, as well as oil absorption at the food surface. During frying, water on the surface of food products is evaporated rapidly under temperatures far above its boiling point, while

water in the center of the food migrates to the surface by concentration and pressure gradients [26]. At the same time, water-soluble food substances move from the interior to the exterior and are leached into the oil [2]. As a result of water escape, crust and pores form on the surface of the food. Crust reduces hydrophilicity and increases oil absorption during cooling stage into the pores developed by moisture loss [26]. This adsorbed oil is the major source of oxidation during storage, and is consumed when the food is eaten. Hence, the quality of the oil at the end of frying is critical for food stability as well as frying efficiency.

2.4 Chemistry of frying

2.4.1 Quality change of oils and products

Frying is a complex process in which many physical and chemical changes occur together in the hot oil and food fried in it. The quality of the oil as the frying medium and the quality of the food produced in it are closely related. Blumenthal established a theory incorporating five stages that describe the relationship between quality changes of food and oil during the frying process [2]. These are shown graphically in Figure 2.

1. Break-In Oil stage: when the oil first comes in contact with the food. The new oil is completely hydrophobic while the food surface is hydrophilic. Therefore, the interaction between food and oil is very poor. At this stage, the food is basically raw, the surface is not crispy, the starch at the center is not gelatinized, and cooked flavors or colors have not developed.

2. Fresh Oil stage: as triacyglycerols break down, small amounts of free fatty acids are released. These fatty acids serve as surfactants that increase the contact between the

oil and the food. Cooking improves at this stage, resulting in crisping of the surface, partial gelatinization of the center, slight browning in color, and increased absorption of oil.

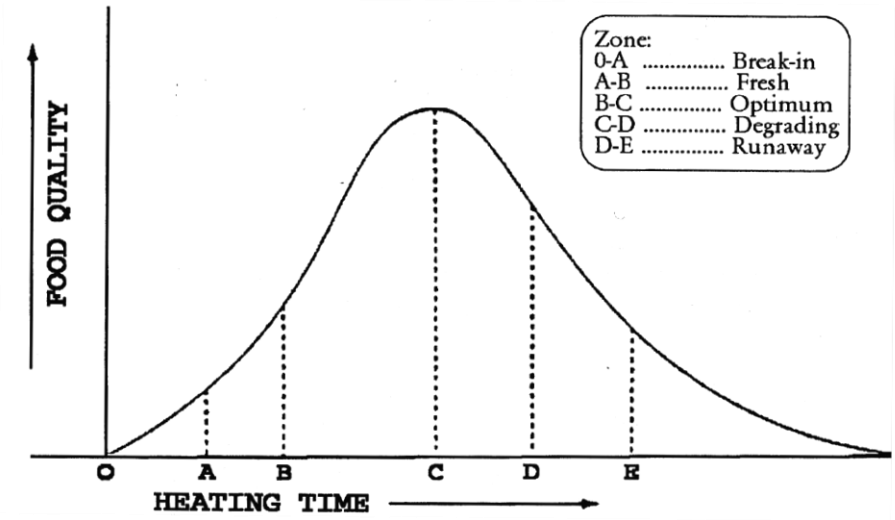


Figure 2. Frying curve developed by Blumenthal [2].

3. Optimum Oil stage: As frying progresses, higher levels of free fatty acids provide sufficient surfactancy for oil to fully contact food surfaces. Heat transfer is most efficient at this point and heat progresses evenly from oil to surface to center of food. Crispiness and golden color is developed on the surface while the center is fully cooked; favorable frying flavors are produced; and oil absorption is at optimum amount at this point. Positive secondary reactions dominate, and oxidation and scission reactions generate flavor compounds and colors.

4. Degrading Oil stage: High heat and extended heating hours increase hydrolysis to the point that negative secondary reactions become dominant. Degrading oil quality results in corresponding deterioration of food characteristics, including darkening and hardening of surface and excessive absorption of oil. Off-flavors and odors begin to develop at this

point.

5. Runaway Oil stage: As the oil degrades still further, the food surface burns, the food becomes very greasy from excessive oil pick-up, and sharp flavor off-notes are produced.

Heating time is not the only factor affecting frying oil quality. Roles of other factors in manipulating quality of the oil, and thus quality of the food will be discussed in more detail in later sections of this Background. Obviously, the ultimate goal is to keep the oil at the optimum stage and prevent it from degrading. To learn how to accomplish this, chemical reactions occurring during frying need to be understood in detail.

2.4.2 Chemical changes involved in thermal degradation of oils – hydrolysis, oxidation, polymerization

2.4.2.1 Hydrolysis

Three major types of reactions have been documented during frying: hydrolysis, oxidation and polymerization. Hydrolysis of ester bonds due to the moisture introduced by foods releases long-chain free fatty acids. Oxidation takes place due to the presence of air, and dimers and polymers are formed by radical recombination to form -C-C-, -C-O-C-, and -C-O-O-C- bonds [1, 5-7]. These three reactions form the core of current frying theory.

Hydrolysis is believed to be the first reaction generating detectable products during frying, which causes major production of free fatty acids [7]. Depending on the extent of hydrolysis, products are fatty acids and, progressively, diacylglycerols, monoacylglycerols, and glycerol (Figure 3). Chromatographic analysis of frying oils with high levels of free fatty acids demonstrated that formation of

monoacylglycerols is minimal, i.e. that hydrolysis tends to completely empty individual glycerol backbones rather than eliminate fatty acids one at a time in sequence on all acylglycerols, as shown in Figure 3.

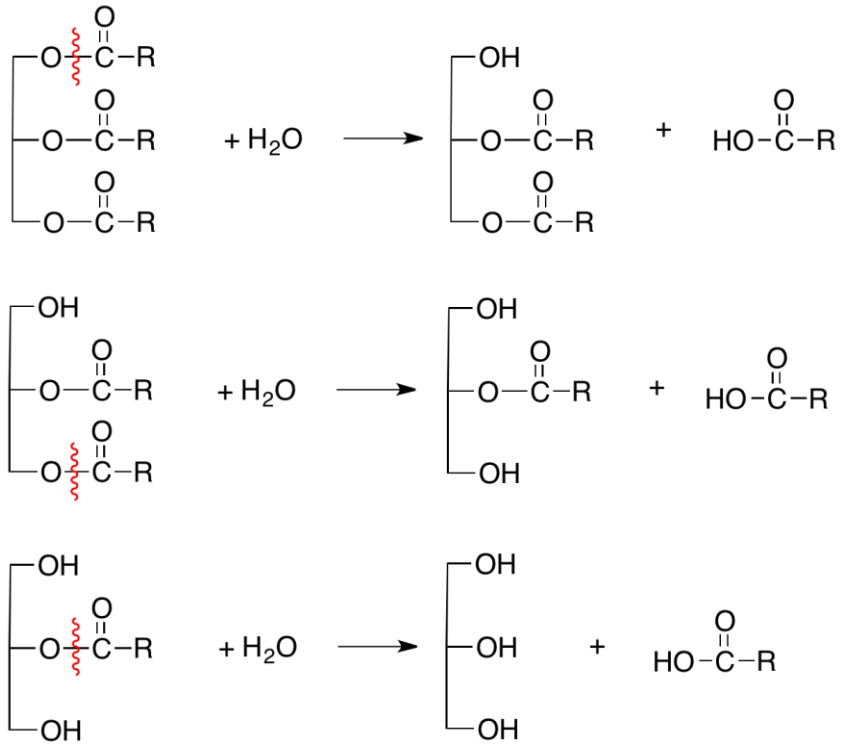


Figure 3. Formation of free fatty acid and diacylglycerol by hydrolysis

Although hydrolysis is one of the simplest reactions, previous research has produced inconsistent results on the formation of hydrolytic products. Some authors found hydrolysis to be the most important reaction during frying, based on detection of high levels of free fatty acids and diacylglycerols [27], while others found fatty acids to be minimal compared to other degradation products, despite high water contents in the food being fried [28, 29]. This inconsistency indicates that variables other than water also play a role in producing the free fatty acids detected in standard analyses.

2.4.2.2 Oxidation

Because the oxygen present in deep-frying reacts with oils, thermal oxidation has been proposed to arise from the same reactions as in lipid autoxidation, but accelerated by Arrhenius kinetics where the rate is doubled for every 10 degrees increase in temperature:

$$k=Ae^{-Ea/RT}$$

or, transformed to eliminate A,

$$\ln(k/k_2) = -Ea/R * (1/T_1 - 1/T_2)$$

where A is a pre-exponential factor related to the frequency of molecular collisions, Ea is the reaction activation energy, R is the universal gas constant (8.314 J/mol K), and T is the reaction or treatment temperature in deg K.

Figure 4 shows one generalized scheme for well-known reactions involved in the autoxidation of lipids. RH here stands for the substrate fatty acid, and H is an α-methylenic hydrogen atom that is easily abstractable due to the influence of the neighboring double bonds. In the initiation stage, an alkyl radical (R•) is formed. In the propagation stage, oxygen adds to the alkyl radical to form peroxyl radicals (ROO•); these radicals, in turn, abstract hydrogens from other molecules to yield hydroperoxides (ROOH) and a new R• radicals that react with oxygen to start a new cycle and propagate the reaction chain. The hydroperoxide intermediates are not stable, but are decomposed by heat to yield reactive hydroxyl radicals (HO•) plus alkoxyl radicals that both abstract H's to propagate and accelerate the oxidation chain and undergo reactions to produce varied products. Reactions are terminated when radicals react with each other to produce non-radical compounds, either volatile or non-volatile, or when alkoxyl radical

scissions or other reactions result in non-radical products.

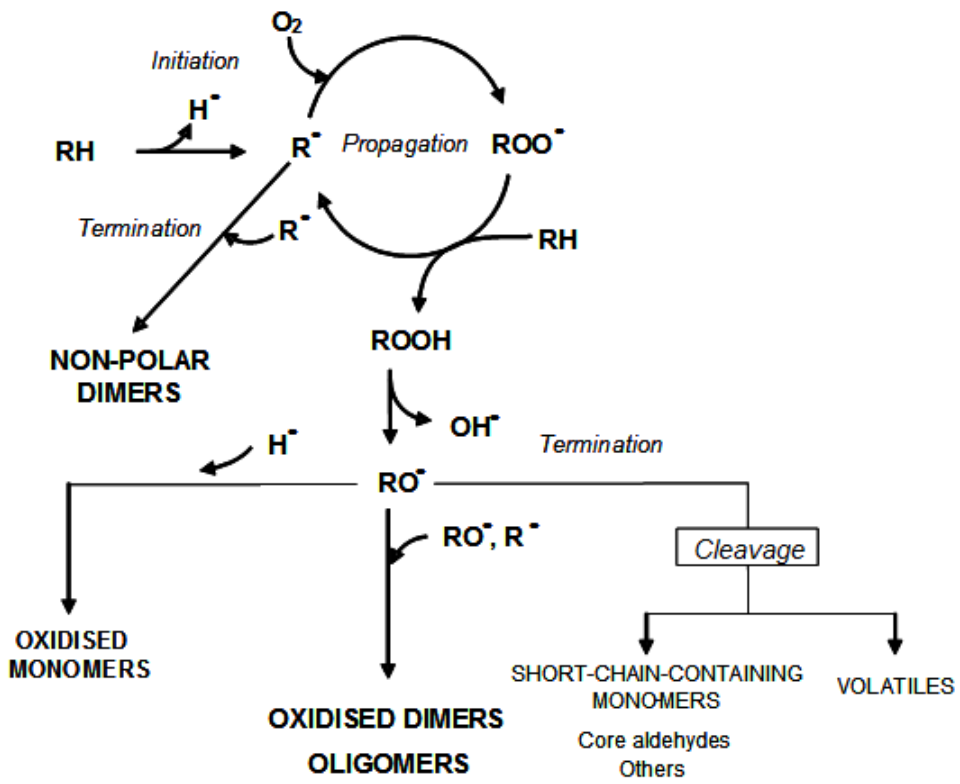


Figure 4. Generalized scheme for autoxidation of lipids**.** From [7].

There are several important characteristics of autoxidation. First, autoxidation requires initiators such as metal, light, heat, enzyme, and etc. Anything that could eliminate these initiators can inhibit autoxidation (e.g. metal chelators). Second, products form in stages, each with unique product classes. In polyunsaturated fatty acids, the first oxidation products are conjugated dienes result from double bond rearrangement after hydrogen abstraction to create the first radical in each chain. Addition of O_2 to radicals generate hydroperoxides immediately afterwards. LOOHs are the major products in the induction period until their rate of decomposition exceeds their rate of formation. Hydroperoxides decompose to a mixture of aldehydes, alcohols, alkanes, and other

compounds. Polymers are late products and do not form in large quantity unless the oil is highly oxidized. Third, the position of –OOH formed is not random due to the preferential abstraction of allylic hydrogens, mostly on the outside positions of double bond systems, i.e. 8-,9-,10-, and 11-hydroperoxides for oleic acid and 9- and 13-hydroperoxides for linoleic acid. Furthermore, the secondary products are specifically related to their parent hydroperoxides. For example, Figure 5 shows the scission reactions of C-9 hydroperoxides of oleic acid; Figure 6 lists characteristic products from scission of 8-, 10-, 11- hydroperoxides.

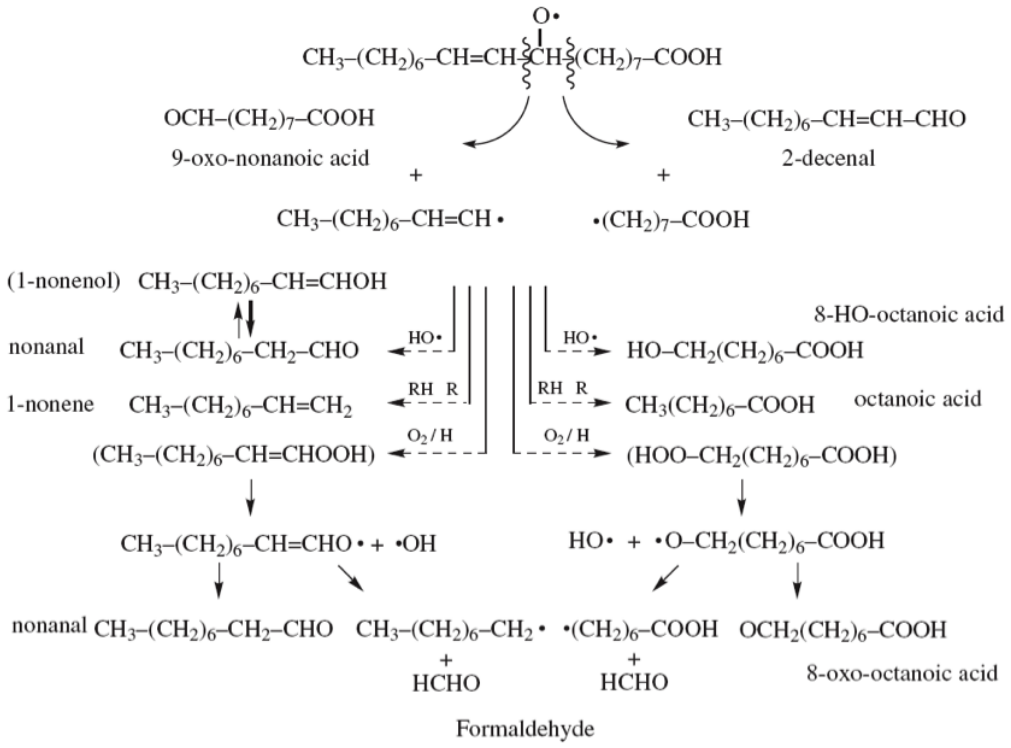


Figure 5. Scission reactions and their product of oxidized oleic acid (9-hydroperoxides). Adapted from Schaich [30].

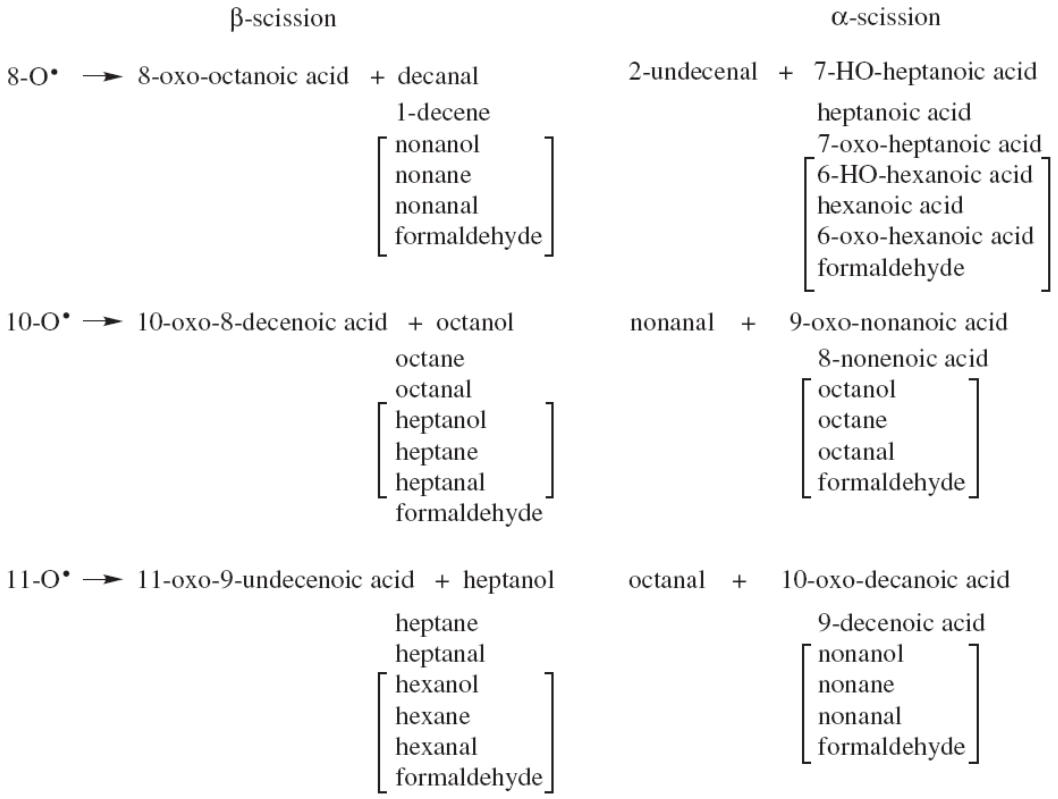


Figure 6. Scission products from 8-, 10-, and 11-hydroperoxides of oleic. Adapted from Schaich [30].

The autoxidation theory of thermal degradation holds that these reactions are not modified at high temperatures, just accelerated by more rapid decomposition of hydroperoxides [31] and presence of higher free fatty acid concentrations [7].

2.4.2.3 Polymerization

Polymerizations are particularly facilitated by high temperatures. Dimerization and polymerization occurs in three ways: 1) radical recombination, 2) addition of radicals to double bonds, and 3) Diels-Alder reactions, a reaction between a double bond and a conjugated diene [32]. The first two are radical reactions.

(1) Radical recombination. When oxygen is limited, allylic radicals recombine with

each other to produce mixtures of dimers. The formation of acyclic dimers from oleate is shown in Figure 7.

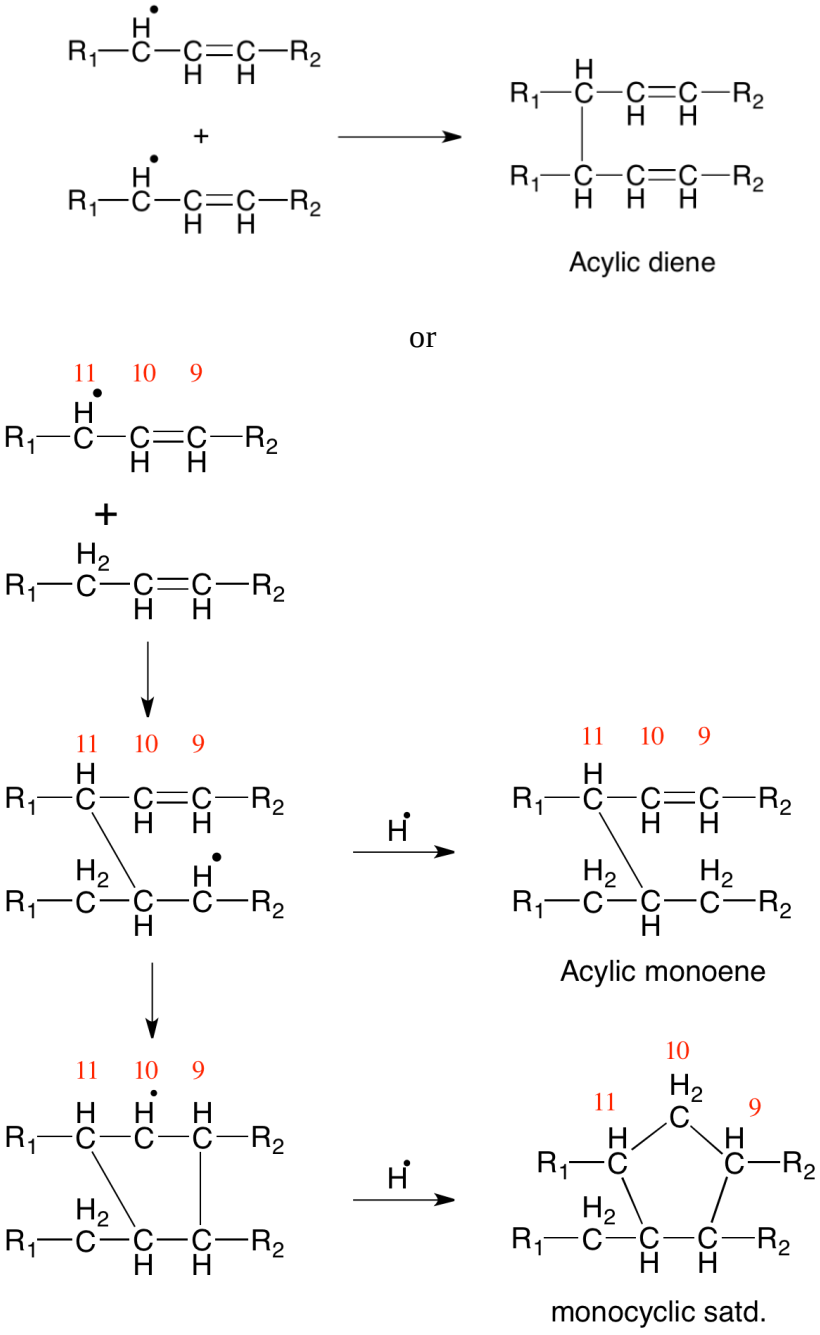


Figure 7. Radical recombination reactions forming dimers in oleic acid. Adapted from Nawar [32].

When sufficient oxygen is present, allylic radicals recombine with alkoxyl and peroxyl radicals, forming ether linkages and peroxide linkages, respectively, as shown for linolenic acid in Figure 8.

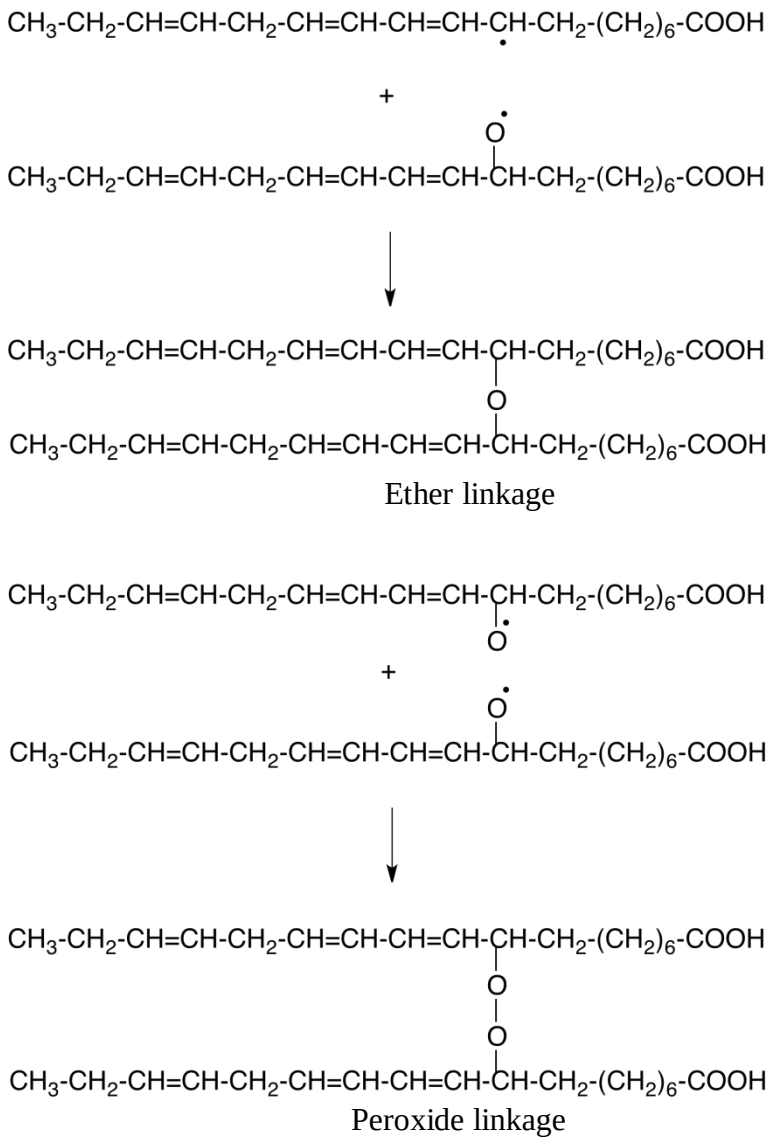


Figure 8. Formation of ether and peroxide linkage polymers in heated linolenic acid (C-9 attack). Adapted from Choe [6].

(2) Addition of radicals to double bonds. Addition of radicals to double bonds yields dimeric radicals, which then may either abstract hydrogens or attack other double bonds to form either acyclic or cyclic compounds. Figure 9 shows the dimerization of linoleic acid by radical additions to double bonds.

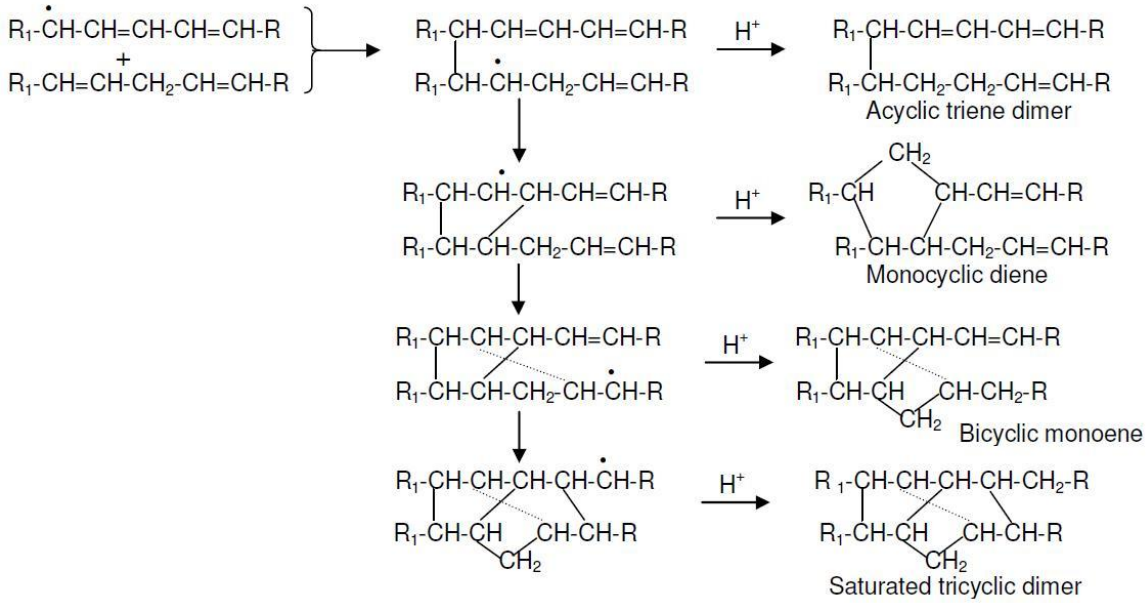


Figure 9. Dimerization of linoleic acid (C-13 attack). Adapted from Schaich [30].

(3) Diels-Alder addition. Oxidation of polyunsaturated fatty acids yields conjugated diene structures. A special form of cyclic dimers is formed by the addition of conjugated dienes to other double bonds to yield intramolecular or intermolecular substituted cyclohexene products (Figure 10).

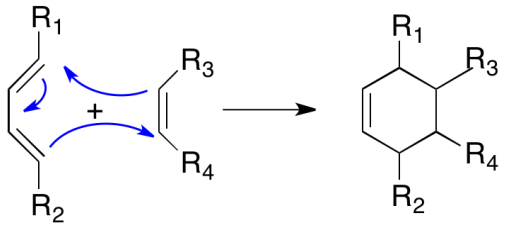
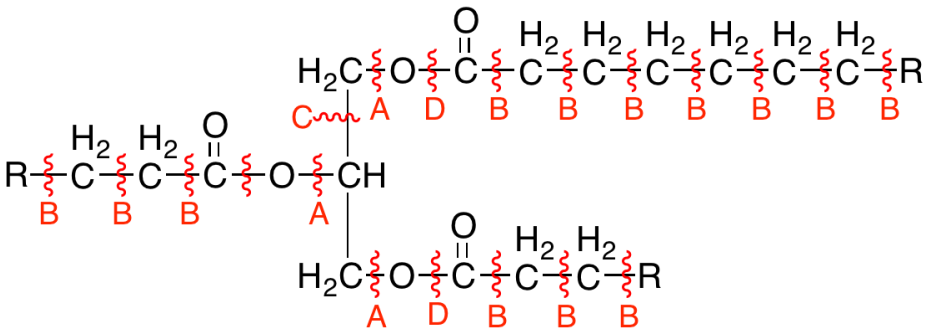


Figure 10. 1,4 Diels-Alder reaction.

2.4.3 Chemical changes involved in thermal degradation of oils - thermal scissions

Nawar's research group at the University of Massachusetts published substantial research documenting thermal scission in fatty acids and triacylglycerols exposed to high temperatures [33-36], yet the role of thermally-induced bond scission to generate free radicals has largely been ignored. Indeed, although the involvement of radicals is recognized, radicals generally have been accepted as arising from accelerated autoxidation or limited to a role in radical recombination reactions occurring under low-oxygen conditions. Both situations beg the question of the initiating source of free radicals. In the autoxidation scheme outlined in the previous section, some initiation process is assumed but none is specified, and consideration of reactions starts with abstraction of hydrogens preferentially from positions where bond energies are low (e.g. allylic hydrogens) [30]. Nevertheless, at high temperatures there is sufficient energy to break almost all kinds of bonds in acyl chains or glycerol. Therefore, thermal degradation should be expected to form products that differ significantly from autoxidation products.

Years of research by Nawar and colleagues [33, 35, 37] have shown that, as a generalization, bond cleavages occur preferentially at the points designated in the triacylglycerol structure in Figure 11, and each cleavage yields a characteristic class of products. Each scission produces two radicals, so large quantities of radicals and an



Cleavage at A → free fatty acids + acrolein (CH_2=CH-CHO)

B → hydrocarbons

C → propanediol and propanediol esters

D → mono- and di-acylglycerols

Figure 11. Proposed bond cleavage points within triacylglycerol molecule and corresponding products. Based on information from **[32]** and references therein.

associated large variety of products are generated within a short time during heating. Thermal scissions have several consequences. First of all, the process depends only on heat and is independent of oxygen, so as long as heat is applied, the degradation continues. This creates a huge radical load that consumes antioxidants and limits their effectiveness in stabilizing frying oils. Second, in contrast to oxidation, major products, even in early stages, are a homologous series of hydrocarbons formed by cleavage at various positions in the acyl chain (position B in Figure 11), followed by H abstraction by alkyl radicals or by recombination of short chain scission fragments. Dimers and polymers are also generated by radical recombinations. Third, scission radicals add oxygen to form terminal peroxyl radicals, which in turn can abstract hydrogens selectively from allylic sites to initiate lipid oxidation chain reactions. At the same time, the hydrogen abstraction generates terminal hydroperoxides that decompose to

oxygenated products with a homologous series of chain lengths. This is in marked contradistinction to autoxidation where products derive from almost entirely from hydroperoxides at specific allylic positions on the parent molecule. For example, a homologous series of alkanes, alkenes, aldehydes and carboxylic acids from very short chain to moderate length have been reported both in model systems as well as in fried foods [33, 38]. Qin [39] used purge and trap methodology and gas chromatography-mass spectrometry to identify homologous series of alkanes, alkenes, aldehydes, and carboxylic acids from C1-C13, along with 2-pentyl furan and smaller amounts of other products in short time heating. This product distribution is not characteristic of lipid autoxidation, although some typical oxidation products were present. Gillat [40] found that aldehydes are produced in notably higher amounts and much greater variety during heating; in particular, short chain aldehydes not produced in autoxidation have been reported for thermal degradation. Acrolein (an oxidation product of glycerol), pentanal, hexanal, heptenal and octenal are commonly detected in heated oils.

2.5 Factors affecting frying oil quality

Although relatively little research has focused on mechanisms of thermal degradation directly, numerous studies have investigated the effects of various catalytic and antioxidant factors on oil quality in deep-frying. These factors cover a wide range, from composition of the oil to components of the food, and from conditions of the frying to the type of fryers. An overview of these studies is presented in the following sub-sections. It is worth noting that results are anything but consistent, and considerable controversy remains regarding influence of these factors on oil stability and quality.

2.5.1 Fatty acid (FA) composition

The common sense notion that oils composed of polyunsaturated fatty acids should have lower frying stability than those composed of monounsaturated and saturated fatty acids has been supported by many studies [41-44]. This is attributed to increasing availability of highly abstractable double allylic hydrogens. Isomers geometry also affect oxidation by altering accessibility of H atoms, with *cis* fatty acids oxidize more readily than *tran* fatty acids. Fatty acid composition is regarded as the most important factors by many researchers, yet there are investigations which found that frying stability could not be predicted by the FA composition alone [45, 46] and was also affected by minor components in the oil, which will be discussed in 2.5.5. Effect of fatty acid composition was not evaluated in this project.

2.5.2 Frying conditions

Long time frying results in accumulation of a wide range of oxidation products including free fatty acids [47], polar compounds [48, 49], dimers [50] and polymers [51], most at high levels. The effect of frying time becomes more significant in used oils that already have some degradation products and surfactants. For example, peroxide values (PV), free fatty acids (FFA) and *p*-anisidine value (*p*-AV) of used palm olein oil were significantly increased ($p<0.05$) with only 3 minutes increase in frying time [52].

High temperatures also accelerate oil degradation and affect fried food quality. When frying temperature of partially hydrogenated soybean oil was increased from 160℃ to 200℃, acid value and polymerization increased notably, and oil color became darker [53]. Sunisa, et al. [52] also found that color and viscosity of used chicken frying oil and

surface color of fried chicken tended to increase with frying temperature and time (170℃ to 190℃).

Intermittent frying generally causes more serious degradation than continuous frying due to increased oxygen solubilization of oxygen in the oil during the cooling period [54]. For example, 25% of linoleic acid was degraded during intermittent frying compared to 5% during continuous frying [55].

2.5.3 Oxygen

Effects of oxygen on frying quality of oils have been studied for decades. Rates of change in viscosity and titratable acidity were found to be directly proportional to the degree of exposure of fat surface to oxygen [56]. Oxygen originally dissolved in oil also affects stability. Canola oil flushed with nitrogen or carbon dioxide has improved stability through reducing oxygen content in the oil [57]. The study suggested flushing oil for 15 min under nitrogen and 5 min under carbon dioxide to increase stability. Dana and others [58] also found nitrogen sparging produced the largest protective effect compared with headspace air flow and water injection.

Previous studies in most cases determined oxygen effect by depleting it completely (for example, using inert gas flushing). It is known, however, that the flavor of fried foods depends on the presence of a certain level of oxidation products. Therefore, it would be meaningful to observe profiles of oxidation products at low oxygen concentrations above zero oxygen. Evaluating low oxygen concentrations is also important from a practical point of view considering the cost and feasibility of applying inert gas in every place along the production line.

2.5.4 Water

Foods complicate the frying process in several ways. One of the most important aspects is continuous release of water from food into oil. The released moisture agitates the oil and promotes hydrolysis. Dornseifer et al. [59] found that adding moisture of 2.5%, 5% and 10% into cottonseed oil increased carbonyl levels compared to dry oil. Moisture also catalyzed production of off-flavors in different kinds of frying oils [60]. However, adding lower amounts of water (e.g. 0.7%) was protective. This protection was attributed to formation of a blanket above the surface of the oil by the steam evolved during frying (or from added water), reducing the amount of oxygen available for oxidation [9, 58]. Steam may also protect the oil by driving out volatile products and possibly quenching free radicals generated during frying [58]. Guler [61] also reported that increase of moisture content in foods slowed down deterioration of olive oil.

Water is an inevitable contaminant in both fresh oil (trace levels) and in frying oil (at high levels) being introduced by food. Average moisture levels in oil during continuous industrial frying of snack products such as potato chips and corn ships is about 1.5-2.0% (privileged information provided by a cooperative company). As mentioned above, several studies have found a protective role of water against oil degradation. This research thus aims to evaluate moisture contents below and above average levels to determine 1) what role water plays given the test oil compositions and 2) the level at which this protective or degradative role comes into effect.

2.5.5 Minor components

Minor components in frying oil include fatty acids, phospholipids, metals, pre-

oxidized compounds, and naturally occurring and intentionally added antioxidants. Levels of these components are reduced after oil refining. However, high levels of phospholipids, metals and other substances may still be re-introduced into frying oil by foods or by contact with processing equipment [62]. Naturally occurring antioxidants such as tocopherols can provide great protection against oxidation for polyunsaturated fatty acids (PUFA) [46]. Sterols, phospholipids and carotenoids are also natural components that are considered to be beneficial with concentration limits applied [60]. Kourimska and others [63] found that addition of 0.1% lecithin significantly reduced concentration of polymers as well as total polars. Lecithin also decreased oxidation of salmon oil heated at 180℃ for 3 hours [64]. Beneficial effects of phospholipids are suggested to be attributed to their synergistic action with tocopherol and chelation with transition metals [62]. In contrast, Yoon and Min [65] reported that in purified soybean oil, phospholipids worked as pro-oxidants by increasing diffusion rate of oxygen from the headspace to the oil. In addition, lecithin has largely been reported to cause excessive foaming as well as darkening of the oil at high temperatures [43, 60].

Free fatty acids are believed to be the first degradation products formed during frying. Many publications note the physical effect of free fatty acids to produce smoke which may lead to a fire hazard [13, 62, 66]; fewer publications address fatty acid effects on chemical alterations. Free fatty acids degrade faster than parent triacylglycerols [7, 67], and their presence has also been shown to catalyze further hydrolysis [62] and oxidation [68]. One proposed mechanism of pro-oxidant activity of FFAs is the acceleration of hydroperoxide decomposition by the carboxyl group [69]. However, O'Brien showed the opposite, that free fatty acids slow oxidations specifically by inducing non-radical

decomposition of the LOOH group.

Metals are notorious for accelerating oxidation. Among them, iron and copper are the most detrimental. Concentrations of metals at which oxidative stability is reduced by 50% are shown in Table 1. It is very clear that copper and iron can affect degradation at lower levels compared to others. Addition of heme ion increases acid values and polymers in oils heated at frying temperatures [53]. Copper is thought to oxidize proteins more than lipids [70], but copper shifts lipid oxidation products and degradation pathways, so its effects on lipids may have been missed. Metals catalyze lipid oxidation by direct initiation, for example via electron transfer to form lipid alkyl radicals or produce reactive oxygen species through metal autoxidation [71]. They also work through indirect initiation and propagation, e.g. by decomposing lipid hydroperoxides to accelerate lipid oxidation [72]. It must also be mentioned that most metal studies were conducted in the context of room temperature autoxidation; fewer studies addressed metal effects on thermal degradation under frying conditions.

Table 1. Concentration of metals at which oxidative stability is reduced by 50%. Adapted from Rossell [3].

Metal	mg/kg
Copper	0.5
Iron	0.6
Manganese	0.6
Chromium	1.2
Nickel	2.2
Vanadium	3.0
Zinc	19.6
Aluminium	50.0

Pre-oxidation accelerates oil degradation during frying by providing initiators for autoxidation chains and foci for secondary interactions. Polar decomposition products of hydroperoxides introduce oxygen into the oil and act as emulsifiers [73]. High temperatures during oil processing generated 1.2% thermally oxidized compounds in RBD (refined, bleached, and deodorized) soybean oil and these compounds accelerated soybean oil oxidation at 55°C [74]. Industrial frying operations found that there are components in used oil which are difficult to remove by filtering. Therefore, identification of the influence of these non-volatile oxidation products on subsequent stability is needed to determine: 1) the extent to which the removal of pre-oxidation products and oil protection during storage can stabilize frying oil against degradation and 2) the difference between early and late oxidation products on frying oil stability.

3. RESEARCH GAPS

Most studies on frying have been oriented towards either performance during use or identifying factors affecting stability of the oil. Very few published studies have investigated thermal degradation mechanisms and kinetics in general or in detail. Current understanding of frying chemistry as autoxidation accelerated by heat was based on class analyses of products, particularly during industrial frying, as well as academic research over long frying times or abusive conditions. Thermal scissions were documented more than three decades ago, yet the role of radicals generated by thermal scission has been largely ignored. In addition, Nawar's work used model systems; we have found no records reporting analysis of thermal scission of edible oils heated under real or simulated frying conditions. Overall, there is very little understanding about the relationship and the specific and relative roles of thermal scission and lipid autoxidation during frying oil degradation. Misinterpreting the contributions of thermal scissions versus autoxidation is very likely one major reason why traditional approaches to stabilizing frying oils, e.g. adding antioxidants, or removing metals and fatty acids, are ineffective. Thus, thermal scission vs autoxidation relationships need to be elucidated.

Previous research on evaluating factors affecting frying typically used long heating times (days to even weeks) in open systems with little or no control over the headspace. Long time heating does not provide useful information for industrial frying, in which turnover time usually averages about 3-8 hours. Furthermore, in actual food operations with real food being fried, more than one of the factors tested are certainly present. Under such conditions multiple factors may interact with each other to exert influences on oil stability that are different from any factor alone. Consequently, it is vital

to evaluate oil changes in oil degradation kinetics or patterns when two or more of these elements act simultaneously, and to determine which factor combinations are synergistic and which may counteract individual actions. For practical application, if a specific factor is found to affect oil only in combination with others factors, stabilization of frying oil can be realized by avoiding co-existence of catalytic multifactors without the extra effort required to eliminate each factor individually. So far, few studies have even considered possible interactions of multiple factors on frying oils, and none have investigated mechanisms involved in the interactions. Indeed, even single factor studies have investigated effects not mechanisms. Thus, it is time to seek more detailed information about reaction mechanisms involved in actions and interactions of the common thought to influence oil stability, and to document potential changes in these effects as oil progresses from fresh to optimum to degrading stages of frying.

Another point worth mentioning is that most frying studies were conducted in open air. Under this condition, sorting out the chemistry is virtually impossible because all volatile products are lost. Clearly new research approaches are needed to elucidate the full sequence of chemical reactions involved in high temperature degradation of oils, information that will provide a new basis for improving stability.

4. HYPOTHESIS AND OBJECTIVES

There is no doubt from Nawar's pioneering studies and from Qin's thesis from this laboratory that thermal scissions do occur during heating and are important in directing product distributions. There is also no doubt that some autoxidation also occurs. The question is, what is the precise role of thermal scission in overall thermal degradation? And what is the role of autoxidation?

4.1 Hypothesis

Based on research reports in the literature and consideration of fundamental free radical reaction chemistry, the hypothesis of this study is: Thermal scissions are critical and integral to thermal degradation chemistry of frying oils.

Since thermal scissions begin as soon as sufficient heat is applied, it is likely that they provide the *ab initio* radicals that drive subsequent degradation processes and are largely responsible for conversions in fresh oil. Thermal scission radicals may a) abstract hydrogens from unsaturated fatty acids to initiate oxidation chains that contribute flavor compounds and browning, b) mediate dimerizations that generate novel products and polymerization that contributes to increased viscosity with longer heating times, and c) generate compounds that are very different than autoxidation products that are not detected in normal analyses, yet play important [if unidentified] roles in the thermal degradation of oils.

4.2 Overall objectives

The ultimate goal of our group's frying project is to provide better understanding

of frying chemistry, which will be useful in improving quality of frying oils as well as in extending shelf life of fried food. As part of the project, this dissertation focused on investigating degradation kinetics and distribution of nonvolatile products (conjugated dienes, hydroperoxides, aldehydes, and free fatty acids) generated during heating, and evaluating roles of common catalysts in directing degradation during early stages of frying.

4.3 Specific objectives

- Use pure frying oil blends typical of those used in the food industry (high oleic sunflower oil: corn oil 60:40) to determine degradation directly without complications from foods.
- Use an Oxipres™ oxygen bomb to provide controlled heating (180 °C, typical frying temperature for potato chips and snacks) in a closed system, in order to trap all compounds generated during heating.
- At the same time, use oxygen bomb to monitor O_2 consumption during oil heating. O_2 consumption data will provide critical degradation information independent of both quantity and variety of degradation products.
- Investigate early degradation (3 hours heating) processes of oils applicable to industrial frying.
- Analyze nonvolatile lipid degradation products (conjugated dienes, peroxide values, carbonyls, free fatty acids) during heating, every 15 minutes up to three hours heating, to determine progression of development of products.
- Investigate independent and interaction effects of factors known to catalyze (or inhibit)

oil degradation during heating -- pre-oxidation, oxygen, water, metals, free fatty acids, phospholipids -- using the same conditions and analyses noted above.

- Integrate results to derive a more accurate picture of thermal degradation versus autoxidation of highly unsaturated oils during heating under conditions modeling industrial frying conditions. Propose an integrated reaction sequence depicting individual roles of thermal scission and autoxidation.

5. MATERIALS AND METHODS

5.1 Materials

Blends of 60% High Oleic Sunflower Oil (NuSun) and 40% Corn Oil were the gift of an industrial frying company. Three types of oils were used in this research to provide different levels of pre-oxidation and endogenous catalytic factors: stripped oil, fresh oil and plant steady state oil. To reduce impurities in stripped oils, oils were passed through powdered sucrose, activated carbon and silica gel at ~5 ml/hr under vacuum. Oils also underwent deodorization at high temperature to remove free fatty acids, sterols and tocopherols. Fresh oils underwent regular refining just before the beginning of this study, and oils were stored and transported under nitrogen. Steady-state oils were blends of 50% fresh oil and 50% used oil pulled from a continuous frying line in a plant. Oils were packaged under nitrogen and shipped in insulated containers with freezer packs using expedited shipment, then stored frozen under argon until use or analysis.

Distearoyl-L-α-phosphatidylcholine was purchased from Sigma Chemical Co. (St. Louis, MO). Stearic acid and Oleic acid were purchased from Nu-Chek-Prep Inc. (Elysian, MN). Iron (II) chloride (anhydrous, 99.9% metal basis), copper (II) chloride (99.9% metal basis), and cobalt (II) chloride were purchased from Sigma-Aldrich, INC. (St. Louis, MO). Peroxy-Safe™, AldeSafe™, and FASafe™ kits were purchased from MP Biochemicals, LLC) (Solon, OH) to analyze hydroperoxides, aldehydes, and free fatty acids, respectively, in heated oils. Iso-octane (HPLC Grade) was purchased from Sigma Chemical Co. (St. Louis, MO). Water used in all tests was doubly-distilled water further purified to 18 megohm (MΩ) resistivity by a four-cartridge Millipore Milli-Q Water Purification System (Millipore, Billerica, MA).

Table 2. Chemical characteristics of stripped, fresh and steady-state oils (information provided by supplier.

	Stripped	Fresh	Steady State
Free fatty acids, wt%	0.03	0.01	0.02
Peroxide Value, meq/kg	0.43	3.13	0.31
p-Anisidine Value, ppm	0.38	2.90	3.00
Tocopherols, ppm			
α	127	554	566
γ	66	169	238
δ	0	0	10
Total	194	723	813
Moisture, wt% (KF)[a]	0.41	0.01	0
OSI[b] (110 C), hrs	4.8	9.5	9.0
Fatty Acid Distribution			
C14	0.03	0.05	0.04
C16	6.51	7.01	6.95
c-C16:1	0.11	0.10	0.09
C17	0.05	0.06	0.06
C18	2.67	2.65	2.80
t-C18:1			
c-C18:1	49.57	51.04	48.10
t-C18:2			
c-C18:2	38.73	37.47	39.94
t-C18:3			
c-C18:3	0.59	0.62	0.46
C20	0.39	0.32	0.38
c-C20:1	0.38	0.34	0.31
C22	0.69	0.30	0.52
C24	0.24		0.30
Total	99.96	99.96	99.95
Metals, ppb			
TI	1.8	1.5	1.6
V	1.9	1.5	1.3
Cr	60.0	11.0	23.0
Mn	2.4	0.9	1.1
Fe	64.0	12.0	33.0
Co	<5	<5	<5
Ni	3.1	2.2	3.3
Cu	16.0	4.5	7.5
Zn	2.8	5.3	3.3
Al	9.8	2.3	4.1

[a] KF=Karl Fischer; [b] OSI=oxidative stability index

5.2 Experimental design

The study was composed of two parts: 1) investigation of fundamental degradation processes in frying oils; 2) investigation of effects of catalytic and antioxidant factors on frying oil degradation kinetics and product patterns.

5.2.1 Thermal degradation kinetics and product patterns in base oils

The experimental design of the degradation process investigation is shown in Figure 12. Twelve oil samples were heated in each run; one sample was removed at designated times during the three hour course of heating and analyzed for nonvolatile degradation products.

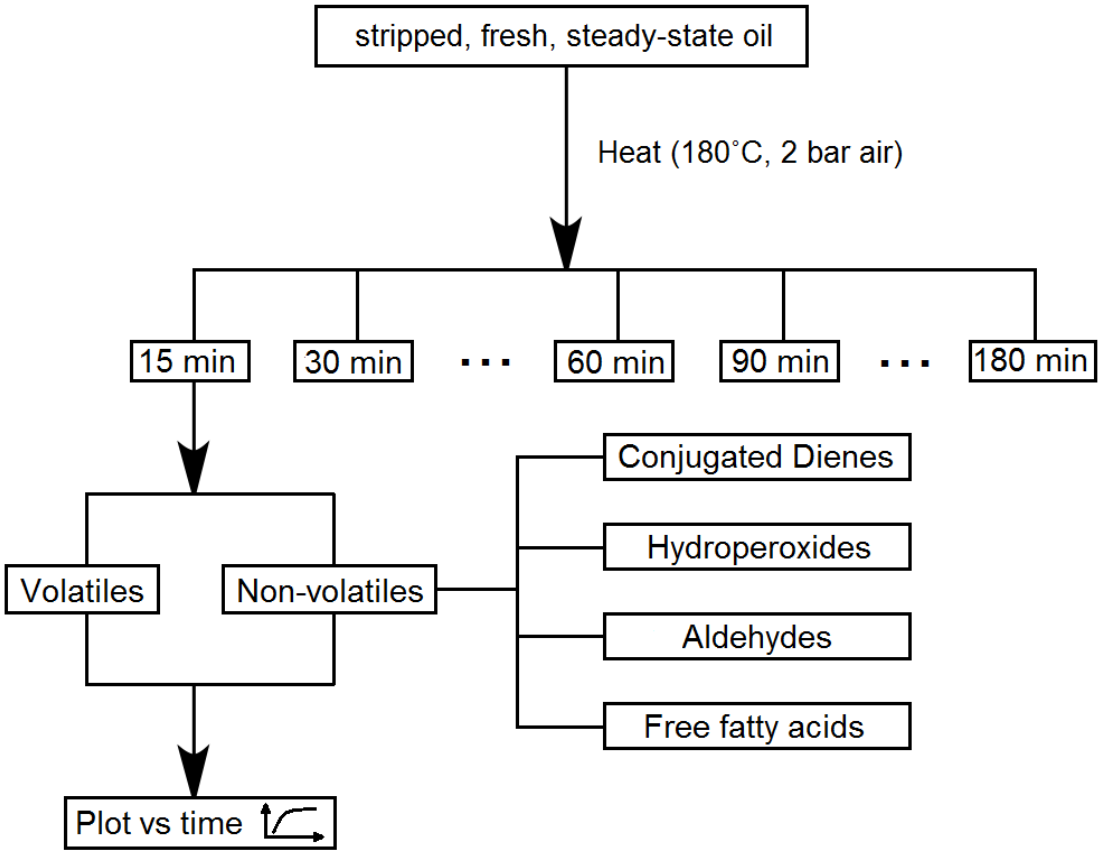


Figure 12. Experimental design for evaluating degradation processes in frying oils.

5.2.1.1 Effects of pre-oxidation on frying oil degradation

Three types of oils (stripped, fresh, steady-state) with different initial oxidation levels were tested to determine effects of pre-oxidation on degradation kinetics and product patterns. Oil samples were heated (180 °C) in oxygen bombs pressurized to 2 bars with gases of different oxygen concentrations (see Section 5.2.1.2). Twelve samples were heated in each series; one sample was withdrawn at each specified time and analyzed for products to follow the kinetics oil degradation, as shown in Figure 13. Detailed methods are provided in Section 5.6.1.

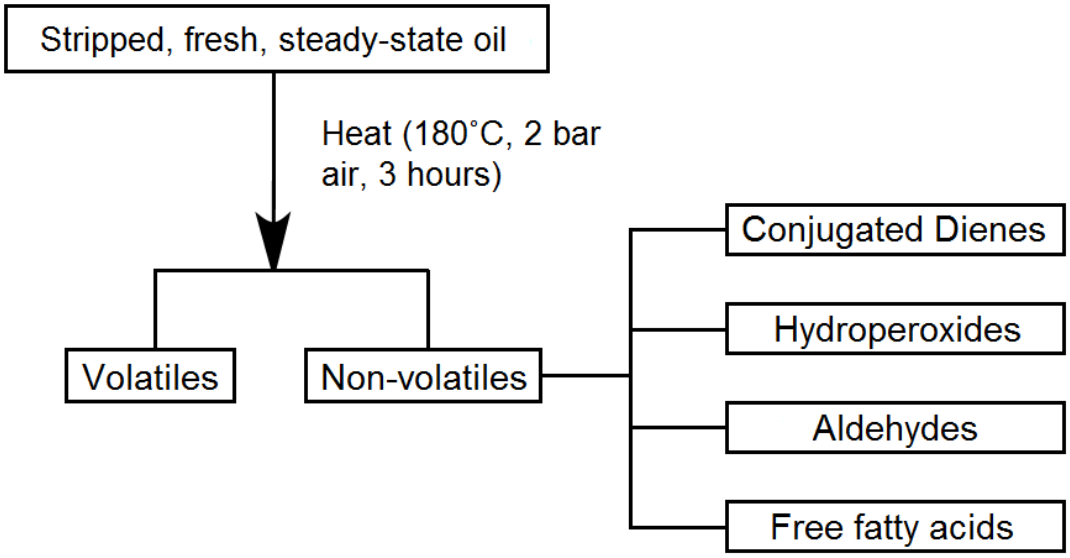


Figure 13. Experimental design for evaluation of effect of pre-oxidation on frying oils.

5.2.1.2 Effects of headspace oxygen on frying oil degradation

Steady-state oil samples were heated (180 °C) in oxygen bombs pressurized to 2 bars with gases of different oxygen concentrations (air, 5% O_2, 2% O_2, nitrogen).

Twelve samples were heated in each series; one sample was withdrawn at each specified time and analyzed for products to follow the kinetics oil degradation, as shown in Figure 14. In one series, oil samples were pre-sparged with argon (Ar) and heated under nitrogen (N_2) to evaluate effects of oxygen dissolved in the oil. Detail methods on product analysis are provided in Sections 5.3 and 5.4.

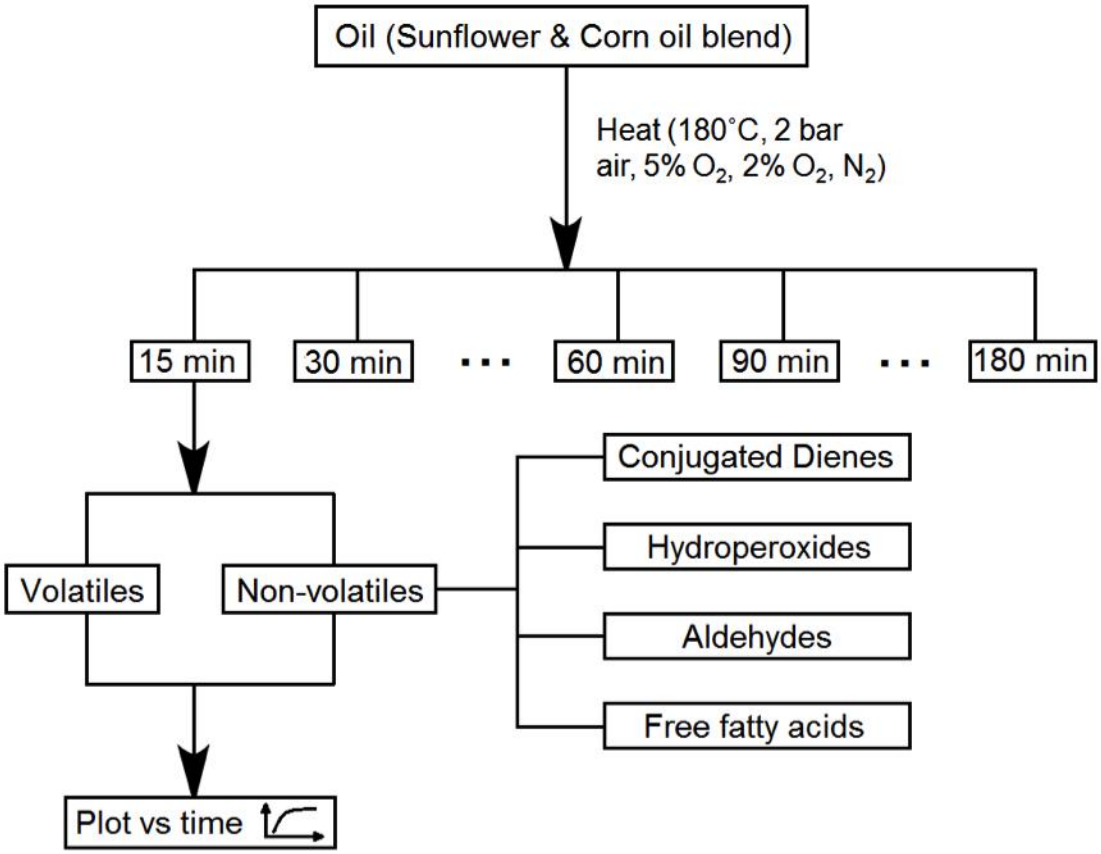


Figure 14. Experimental design for evaluating O_2 effects on frying oil degradation.

5.2.2 Effects of potentially catalytic factors (water, metals, fatty acids, phospholipids) on frying oil degradation

Different levels (below and above common concentrations found in frying oils) of

water, metals, free fatty acids, and phospholipids were added to steady-state oil, and the oil samples were heated under 2 bars air for 3 hours. Factors were tested alone and in the following 2-way and 3-way combinations: metal + water, fatty acid + water, phospholipid + water, fatty acid + metal, phospholipid + metal, fatty acid + metal + water, phospholipid + metal + water. Detail methods on product analysis are provided in Sections 5.3 and 5.4.

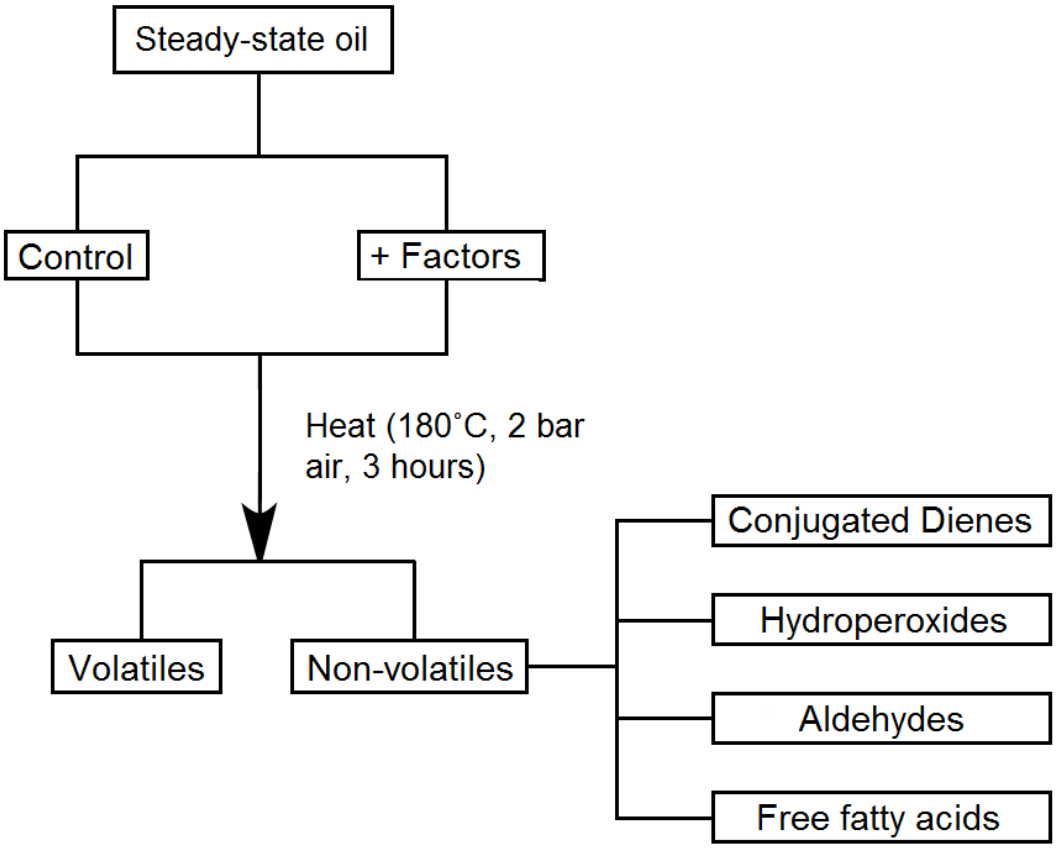


Figure 15. Experimental design for evaluating effects of water, metals, fatty acids, phospholipids on frying oil degradation.

5.3 Determination of O_2 consumption by Oxipres™ oxygen bombs

5.3.1 Oxipres™ oxygen bomb instrumentation and operating conditions

The ML Oxipres™ (MIKROLAB, Denmark) oxygen bomb was designed to monitor headspace oxygen consumed during oxidation of oils and fats, as well as materials containing oils or lipids. Following accelerated shelf life principles, the Oxipres™ operates at elevated temperature (°C**)** and pressure (in bars, where 1 bar = 0.1 MPa = 14.504 psi). Each Oxipres unit is composed of a control base and a two-position heating block (Figure 16). Temperature and pressure are set manually for each sample vessel. The control unit connected to a computer provides precise control of experimental heating cycles and records pressure data. Six of these units were combined to provide capability of running 12 samples simultaneously.

Figure 16. Oxipres oxygen bomb system with three samples running in individual positions of the control units. A gas supply and computer connect to each oxygen bomb cell.

Oil samples were weighed into specially-designed glass flasks that have three indentations in the lip to allow exchange of gas (Figure 17). Flasks were then covered with a glass lid to prevent contamination of the oil from falling particles and placed in the stainless steel pressure vessels. Each vessel base was sealed by a metal cap with a pressure transducer embedded on the top (Figure 18). Data cables link each vessel sensor to the computer for recording oxygen consumption. Each pressure vessel was connected in turn to the supply gas tank through a filling station that controlled the gas pressurization process (Figure 19), pressurized to target pressure with the test gas desired, and then transferred to a heating block preheated to desired temperature to start the experiment. Computer data recording was activated simultaneously.

(a) (b) (c)

Figure 17. (a) An Oxipres™ sample flask; (b) oil deposited in the flask; (c) flask placed in pressure vessel. Glass lid is place on top of flask during heating to prevent contamination to oils.

Figure 18. An Oxipres™ pressure vessel with pressure transducer (on the top) and signal transmitting lines.

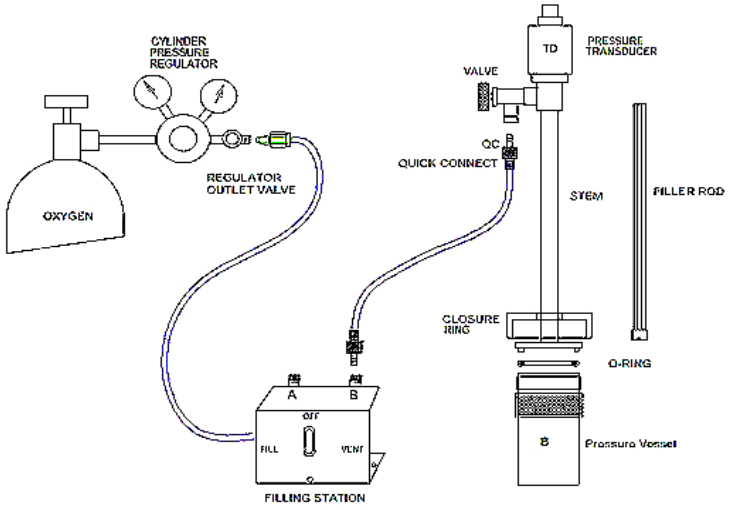


Figure 19. Scheme for pressurizing Oxipres™ pressure vessels.

5.3.2 Oxipres™ operating conditions

Different conditions and sample sizes have been reported for studies of oxidative

stability using the Oxipres™ or other oxygen bombs. Liang and others [75] heated 50 grams of sample continuously under 50 psi (≈ 3.45 bar) pure oxygen at 99°C for 24 h in their study of four different stability methods for lard and tallow. Trojková and others [76] tested different weights of rapeseed oil in an Oxipres oxygen bomb under different pressures and temperatures and suggested using 10-20 g sample sizes, applying 0.5Mpa (5.0 bar) pressure and heating samples at a temperature of 100 °C or slightly higher.

Preliminary experiments were carried out to determine Oxipres™ optimum operating conditions for frying degradation. Considerations included actual frying conditions, and sensitivity of the oxygen bomb. Results showed that the following conditions provided most accurate results with fewest complications:

(1) Sample size: 10 gram. Larger sample sizes consumed too much space in the flasks, resulting in oxygen limitation, while samples sizes smaller than 10 g had a high surface: volume ratio that caused rapid burning and excessive degradation.

(2) Heating temperature: 180°C. Normal frying temperatures range from 180-200°C [77]. 180°C is typical of industrial frying temperatures for potato products and other snacks, which were being modeled in this study.

(3) Heating time: 3 hours for standard screening. Continuous industrial frying of snack products has a rapid turnover rate, varying from 5-10 hours [24]. After three hours heating in the Oxipres™, oil degradation was comparable to oils in continuous industrial frying operations. Heating times of 24 hours and longer used in many frying studies caused very extensive degradation and burning, so results were not relevant to actual frying operations.

(4) Bomb pressure: 2 bars air for standard operations. At all pressures, sufficient oxygen was available to fully oxidize more TAGs than were present in 10 g oil. At the 5 bars pressure recommended by the manufacturer, whether oxygen or air, oxidation rates and extents far exceeded degradation normally seen in frying so these conditions were unacceptable for modeling practical frying or theoretical thermal degradation. Preliminary experiments showed that oxygen consumption decreased linearly as bomb pressure was decreased. However, sensor response was compromised at pressures lower than 1 bar. Thus, 2 bars pressure provided the best compromise between sensitivity and appropriate reaction conditions.

5.3.3 Interpretation of oxygen bomb data

The Oxipres™ software records oxygen pressure (in bars) continually from the point when heating of the oil begins. A typical oxygen bomb curve is divided into four regions, all of which provide different information about the degradation processes occurring in heated oils (Figure 20). Time to O_2 peak and peak pO_2 reflects oxidizability of the test oil. As the cells are heated, pressure naturally rises to a pressure determined by the combined gas law PV=nRT at a rate determined primarily by heat transfer. However, when samples are oxidizing rapidly, both the time to peak and the peak pressure are reduced because oxygen consumption begins immediately. The peak pressure thus reflects an equilibrium between rise in temperature and consumption of oxygen.

Net O_2 consumption (bars) was calculated by subtracting the final pO_2 from peak pO_2 (Figure 20), and bars oxygen were converted to mmols O_2/mol TAG, using the combined gas law (PV=nRT) and average TAG molecular weight of 885 g/mol

(calculated from the proportional composition of fatty acids in the 60:40 oil blend). This value was the net result of O_2 consumed during heating, O_2 returned to the headspace by reactions such as the Russell mechanism for recombination of peroxyl radicals [78],

$$LOO^{\bullet} + {}^{\bullet}OOL \rightarrow L\text{-}OOOO\text{-}L \rightarrow 2\ LO^{\bullet} + O_2$$

and potentially also release of volatile products into the headspace. Headspace analysis of volatiles collected from Oxipres flasks, conducted by Ilona Repko [79], verified that volatile products contributed negligible pressure to the headspace compared to O_2 consumption (Table 2) and legitimized the use of pressure difference as a measure of O_2 consumption. Oxygen consumption is the most straightforward index to reflect degree of oxidation since it reflects the initial radical oxygenation step that always occurs in radical chain reactions, i.e.

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$$

and is independent of subsequent product formation or degradation pathways. O_2 consumption rates calculated from the maximum slope in a 10-minute segment (mmols O_2/min) provide kinetic information about degradation processes under different conditions or with different materials (Figure 20).

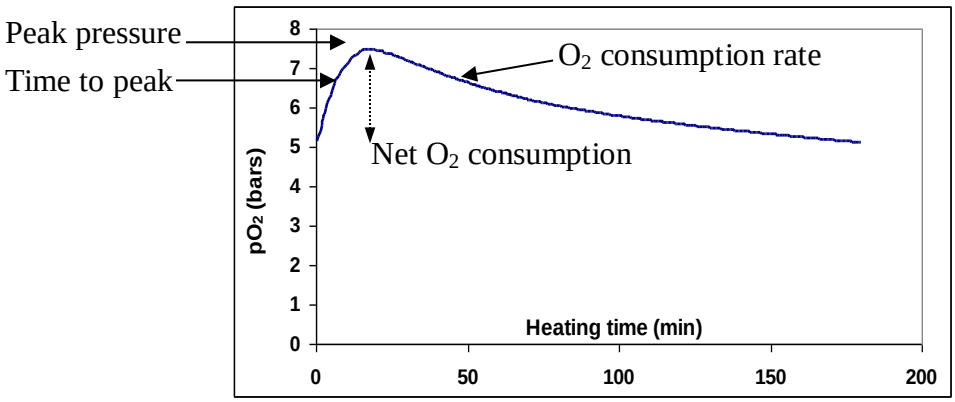


Figure 20. Typical Oxipres oxygen consumption generated when oils are heated to high temperatures (180 °C) under 5 bars oxygen pressure. Slopes and oxygen consumption decrease at lower temperatures and oxygen pressures.

Table 3. Comparison of total volatiles released versus O_2 consumption. Values presented as examples were selected randomly from different runs reported in Repko's thesis [79].

ΔP in Oxipres curve (bars)	n volatiles (mols)	n volatiles converted to P (bars)	Volatiles as % O_2 consumption
0.2234	0.0206	0.0048	2.15%
0.3284	0.0159	0.0037	1.13%
0.2711	0.0197	0.0046	1.70%

5.4 Analysis of degradation products

Four non-volatile degradation products commonly used as lipid degradation indices were measured in this study: conjugated dienes, peroxide values, aldehydes and free fatty acids. In autoxidation, conjugated dienes and hydroperoxide are initial products whose degradation generates secondary oxidation products such as aldehydes (Figure 21). Free fatty acids have been widely reported as the first products of thermal degradation, caused by hydrolysis accelerated by heat. Degradation patterns of these four products, supplemented with volatile products trapped from the oxygen bomb and analyzed by gas chromatography , were used to distinguish thermal degradation from autoxidation processes.

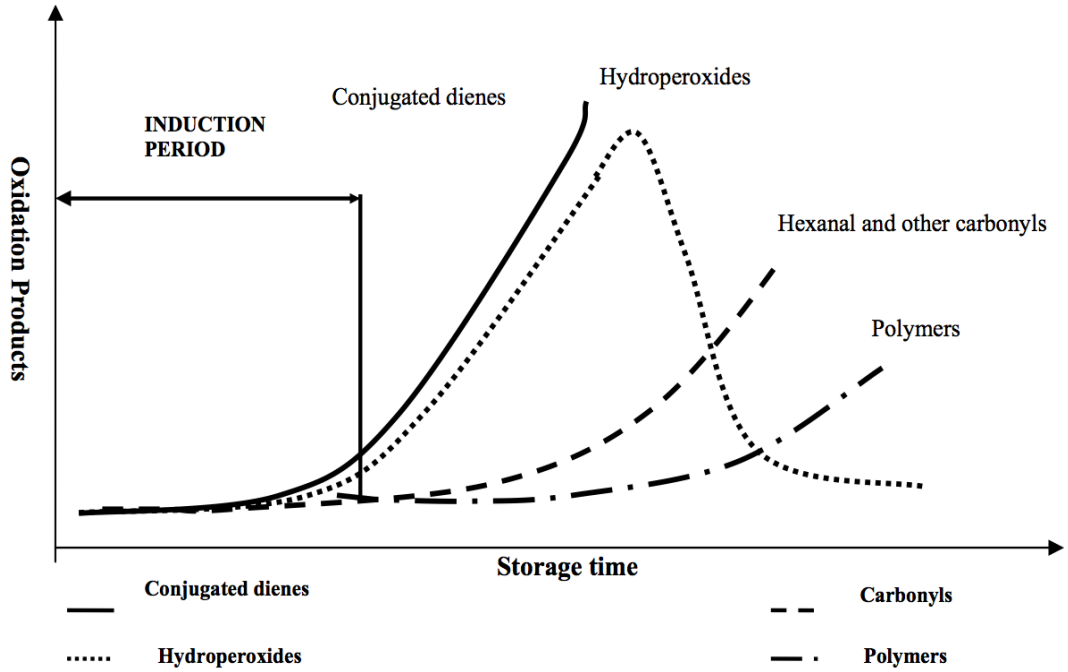


Figure 21. Expected time sequence for development of products in lipid autoxidation [80]

5.4.1 Conjugated Dienes

Conjugated dienes of oils before and after heating were analyzed by a modification of AOCS procedure Ti 1a-64. 30 µl of oil were dissolved in 10 ml of iso-octane (HPLC grade) in a 15 ml test tube; an aliquot was transferred to a quartz cuvette and optical absorbance of the solution was measured at 234 nm against an iso-octane blank using a Varian Cary 50 UV/Vis Spectrophotometer (Varian Inc., USA). Sample solutions in the cuvette were diluted further if absorbance values were higher than 1 where instrumental accuracy decreased. Concentration of conjugated dienes (mmols) was then calculated from Beer's Law using the measured absorbances and a molar extinction coefficient of 26,000 L $mol^{-1}cm^{-1}$. This value was converted to mmols/mol TAG using Beer–Lambert law.

5.4.2 Peroxide value

Peroxide values of fresh and heated oils were determined using the PeroxySafe™ Standard Assay (MP Biomedical, Solon, OH) which is based on the Fe^{2+} reaction with LOOH, followed by trapping of the Fe^{3+} released by xylenol orange [81]. The assay was calibrated every two weeks using calibrators provided in the kit. Because the xylenol orange reaction response is extremely sensitive (detects nmols LOOH) and the reporter complex is bleached by excess hydroperoxides [81], oil samples were diluted with PeroxySafe™ Prep Reagent before each analysis. The dilution required depended on degree of degradation, and varied with different samples. Samples were diluted to read within detection range of the test kit. Detection range for PeroxySafe™ is 0.05-0.5 meq peroxide/kg fat. 25 µl of diluted oil sample, 1000 µl of PeroxySafe™ Reagent A, 100 µl Reagent B and 160 µl Reagent C were micropipetted into 10mm × 75mm test tubes and vortexed. The mixture was incubated at room temperature (25 °C controlled in a heating block) for 15 minutes and absorbance at 570 nm was recorded using the SafTest optical analyzer. Peroxide values in units of meq peroxide/kg fat reported by the PeroxySafe software were converted to mmols/mol TAG to be able to compare all products on a common basis that more clearly reflected reaction extent.

5.4.3 Aldehydes

Aldehyde concentrations were determined using the AlkalSafe™ Standard Assay (MP Biomedical, Solon, OH), reportedly based on indole complexation of aldehydes. This reaction was designed to provide a non-toxic replacement for the toxic *p*-anisidine assay which preferentially detects unsaturated aldehydes [82]. However, we observed

comparable reactions with decanal, *trans*-2-decenal, 2,4-decadienal, and shorter chain saturated aldehyde standards in the AlkalSafe™ standard assay. Therefore, the AlkalSafe™ Assay does not distinguish specific classes of aldehydes, but does give broad-based information on oxidation and is good method for relative value.

The assay was calibrated every two weeks using calibrators provided in the kit. As with the peroxide assay, oil samples were diluted in AlkalSafe™ Prep Reagent to a range of concentrations before analysis. Dilution factors were determined based on detection range of 8-64 nmol/ml for AlkalSafe™ (detailed see 5.4.2). 70 µl of diluted oil sample, 1000 µl of AlkalSafe™ Reagent A and 250 µl Reagent B were micropipetted into test tubes and vortexed. The mixture was incubated at room temperature (25 °C controlled in a heating block) for 20 minutes and optical absorbance at 550 nm was recorded using the SafTest optical analyzer. Aldehyde concentrations reported as nmol/ml reaction by the SafeTest software were converted to mmols/mol TAG to be able to compare all products on a common basis that more clearly reflected reaction extent.

5.4.4 Free fatty acids

Free fatty acid concentrations were determined using the FASafe™ Standard Kit (MP Biomedical, Solon, OH). The mechanism and reaction basis of this assay was not given by the supplier, but screening tests with stearic, oleic and linoleic standards showed that this assay measures both saturated and unsaturated fatty acids. As with the other SafTest assays, calibration was conducted every two weeks using calibrators provided in the kit. Oil samples were diluted in FASafe™ Prep Reagent to a range of concentrations before analysis. Dilution factors were determined based on detection range of 0.04-2 % fat for FASafe™ (detailed see 5.4.2). 50 µl diluted sample, 1000 µl of FASafe™

Reagent A and 100 µl Reagent B were micropipetted into test tubes and vortexed. The mixture was incubated at 37-44°C (controlled in a heating block) for 10 minutes, then optical absorbance at 550 nm was recorded using the SafTest optical analyzer. Fatty acid concentrations were calculated by the SafeTest software and reported as % FFA.

5.5 Methods to determine degradation processes in heated oils

Eight 10 gram replicates of oil samples (fresh, stripped, steady-state) were weighed into Oxipres cells as described above, sealed, pressurized to 2 bars with air, then heated to 180 °C and held for up to three hours. Samples were removed for analysis every 15 min (up to 60 min) where changes were occurring rapidly, or every 30 min in later stages (60-180 min) where degradation was occurring more slowly. At the end of the specified heating periods, before opening the vessel, the headspace was vented through a thermal desorption trap connected to the gas inlet to collect volatile products. Two types of thermal desorption traps were used: Tenax-Carboxen (1:1, 50 mg each) traps for dry samples and 100% Tenax for samples with water. Volatiles collected in the traps were then analyzed by GC-MS [79]. The gas inlet was then closed again, and the pressure vessel was removed from the Oxipres heating block and allowed to cool to room temperature before opening. Heated oils were immediately transferred to sample storage vials, flushed with argon, and analyzed for conjugated dienes, hydroperoxides, aldehydes, and free fatty acids as described in Section 5.4. Data were plotted against heating time to reveal patterns of product development.

5.6 Effects of catalytic factors

All three oils (fresh, stripped, steady-state) were used in the investigation of factors commonly reported to catalyze or inhibit degradation of frying oils. Systematic evaluation of single factors, 2-way combination and 3-way combination was conducted in steady-state oil, whereas only single factors were evaluated in fresh and stripped oils. Therefore, with the exception of pre-oxidation effect in which we looked at the difference between three oils, results on effects of all other factors under discussion in this dissertation are from tests using steady-state oil.

5.6.1 Individual factors

5.6.1.1 Pre-oxidation level

To determine effects of pre-formed hydroperoxides and carbonyl products on lipid oxidation in frying oils, three oils with increasing pre-oxidation levels were tested: stripped oil, fresh oil and steady state oil from a commercial frying plant. For each oil, 10 gram samples were heated in the Oxipres™ at 180 °C under 2 bars air for three hours. Oxygen consumption and maximum oxidation rates were determined. Volatiles were trapped and analyzed by GC-MS [79]. Non-volatile products including conjugated dienes, hydroperoxides, aldehydes, and free fatty acids were evaluated at the end of the heating.

5.6.1.2 Oxygen

10 grams of samples of steady-state oil were heated in oxygen bomb vessels pressurized with a range of oxygen concentrations (2 bars of air, 5% oxygen, 2% oxygen, or nitrogen) to determine oxygen effects on frying oil degradation. One series of oil samples was sparged with argon to deplete dissolved oxygen then heated under nitrogen. Samples were removed every 15 minutes for the first hour, then every 30 minutes

thereafter. Oxygen consumption and maximum oxidation rates were determined, and non-volatile products were determined throughout the course of heating. At the end of heating, volatiles were trapped and analyzed by GC-MS.

5.6.1.3 Water

0.5% and 2% Milli-Q water (18 MΩ) was added to ten grams of steady-state oil in Oxipres flasks, the flasks were flushed with argon and sealed with a rubber stopper, then the mixture was vortexed for 10 minutes to disperse water in the oil. The rubber stoppers were replaced with standard Oxipres glass lid, and all flasks were then immediately transferred to the oxygen bomb, pressurized, and heated for three hours under 2 bars air. Oxygen consumption was followed and oxidation rates were determined. At the end of heating, volatiles were trapped and analyzed by GC-MS, then oils were cooled and immediately analyzed for non-volatile products. 10 grams samples of steady-state oil with no added water served as a control.

5.6.1.4 Metals

500 ppm stock solutions of $FeCl_2$, $CuCl_2$, and $CoCl_2$ were prepared using 18 MΩ Milli-Q water. Before each experiment, stock solutions were diluted 1:10 and 1:100 to 50 ppm and 5 ppm respectively. Aliquots of 0.2 g stock and diluted solutions were deposited at the bottom of the flask using disposable pipettes, and the water was evaporated completely under argon. Ten grams of steady-state oil were added to each flask to give final metal concentrations of 10ppm, 1ppm, and 100ppb. Each oil sample was stoppered and vortexed for 10 minutes as described in 5.6.1.3 to disperse metals. O_2 consumption was followed and degradation products were analyzed at the end of heating. 10 grams of steady-state oil with no added metals served as a control.

5.6.1.5 Free fatty acids

Effect of two fatty acids were evaluated: stearic acid (saturated) and oleic acid (unsaturated). 0.005, 0.025, and 0.050 g of the fatty acid were weighed and deposited on the bottom of Oxipres flasks. Ten grams of steady-state oil were then added to reach final fatty acid concentrations of 0.05%, 0.25% and 0.5%. Samples were stoppered, flushed briefly with argon, vortexed to disperse the fatty acids, then heated for three hours at 180°C as described above. O_2 consumption and degradation products were analyzed at the end of the heating period. 10 g samples of steady-state oil with no added fatty acids served as a control.

5.6.1.6 Phospholipids

0.002 g and 0.005 g distearoyl L-α-phosphatidylcholine were weighed and deposited at the bottom of Oxipres flasks. Ten grams of steady-state oil were added to each flask to reach final concentrations of 0.02 % and 0.05 % phospholipid. The flasks were stoppered, flushed briefly with argon, vortexed to disperse the phospholipid, then heated as described above. O_2 consumption and degradation products were analyzed at the end of the heating period. 10 g samples of steady-state oil with no added phospholipids served as a control.

5.6.2 Combinations of factors

5.6.2.1 Metal + H_2O

500 ppm, 50 ppm and 5 ppm of $CuCl_2$ aqueous solution were prepared as described in 5.6.1.4 using 18 MΩ Millipore Milli-Q water. 0.2 grams of each diluted

solution was deposited at the bottom of the flask using disposable pipets. After that, ten grams of steady-state oil was added into each flask, giving final concentrations of 10ppm, 1ppm and 100ppb for metal, and 2% for water. Flask headspaces were flushed with argon and sealed with a rubber stopper. The mixture was then vortexed for 10 minutes. Rubber stoppers were replaced with a glass lid, and all flasks were immediately transferred to the oxygen bomb and heated under 2 bars air. Oxygen consumptions were followed and oxidation rates were determined. At the end of heating, volatiles were trapped for GC-MS analysis. Oils were cooled down and immediately analyzed for non-volatile products. Ten grams of steady-state oil with no added metals and water was heated as control. Also, considering the significant contribution of water to bomb pressure, a "water control" was also run by heating oil with 2% water added.

5.6.2.2 Fatty acid + H_2O

0.005 g, 0.025 g and 0.050 g oleic acid were weighed and deposited at the bottom of Oxipres flasks. 0.2 grams of 18 MΩ Milli-Q water was also added into the flask. Ten grams of steady-state oil was then added to reach final concentrations of 0.05%, 0.25% and 0.5%, respectively, for oleic acid and 2% for water. Oil and added factors were mixed and heated as described above. An oil control and a water control were run to compare with treated samples.

5.6.2.3 Phospholipid + H_2O

0.002 g and 0.005 g distearoyl L-α-phosphatidylcholine was weighed and deposited at the bottom of Oxipres flasks. 0.2 grams of Milli-Q water was also added. Ten grams of steady-state oil were then added to give final concentrations of 0.02% and 0.05%, respectively, for phospholipid and 2% for water. Oil and added factors were

mixed and heated as described above. An oil control and a water control were run with treated samples.

5.6.2.4 Metal + Fatty acid

100 ppb, 1 ppm and 10 ppm $FeCl_2$ were deposited in Oxipres flasks as described in Section 5.6.1.4. 0.5% Oleic acid was added afterwards. Ten grams of steady-state oil were added and the mixture was vortexed under argon for 10 min. Mixtures were then heated in the oxygen bomb. Oxygen consumptions were followed and oxidation rates were determined. At the end of heating, volatiles were trapped for GC-MS analysis and non-volatile products were analyzed. Oil alone was heated as a control.

5.6.2.5 Metal + Phospholipid

100 ppb, 1 ppm and 10 ppm $FeCl_2$, and 0.05% distearoyl L-α-phosphatidylcholine was added. Ten grams of steady-state oil were added afterwards and the mixture was vortexed under argon for 10 min. Oils with added factors were heated and analyzed as described above. Oil alone was heated as a control.

5.6.2.6 Metal + Fatty acid + H_2O

100 ppb, 1 ppm and 10 ppm $FeCl_2$ was added as described in Section 5.6.1 but without drying the water. 0.5% Oleic acid was also added. Ten grams of steady-state oil were added afterwards and the mixture was vortexed. Oils were heated and degradation products were analyzed. Pure oil and oil added with 2% water were heated as oil control and water control, respectively.

5.6.2.7 Metal + Phospholipid + H_2O

100 ppb, 1 ppm and 10 ppm $FeCl_2$ were added without drying the water. 0.05% distearoyl L-α-phosphatidylcholine and then ten grams of grams of steady-state oil were

added. Mixtures were vortexed and heated. O_2 consumption and degradation products were analyzed. Pure oil and oil + 2% water were heated as oil control and water control, respectively.

5.7 Data analysis

All experiments were performed at least in duplicates. Average values were presented in all tables and figures. A t-test was performed using SAS (Cary, NC) statistical software to determine whether differences in oxygen consumption (amount and rate), conjugated diene value, peroxide value, aldehyde and free fatty acid value of the oils subjected to different treatments were statistically significant.

6. RESULTS

6.1 Development patterns of individual degradation products

Degradation processes in stripped, fresh, and steady-state oils were investigated. General patterns of degradation products are presented in this section (6.1); more details of difference between the three oils will be discussed in Section 6.2.

6.1.1 Conjugated dienes

Conjugated dienes, the initial products in autoxidation, are formed during the oxidation of unsaturated fatty acids with two or more double bonds to achieve a stable resonance structure. In the case of linoleic acid oxidation, for example, hydrogen abstraction is thought to occur preferentially from doubly allylic C11, which has the lowest C-H bond energy. The odd electron delocalizes across the five carbon system from C9 to C13, but electron density is concentrated at C11, making C9 and C13 electron deficient. When oxygen adds to these outer positions of the 1,5-diene system, an electron pair moves over one carbon to form a stable conjugated diene with a peroxyl radical at C9 or C13 [83] (Figure 22).

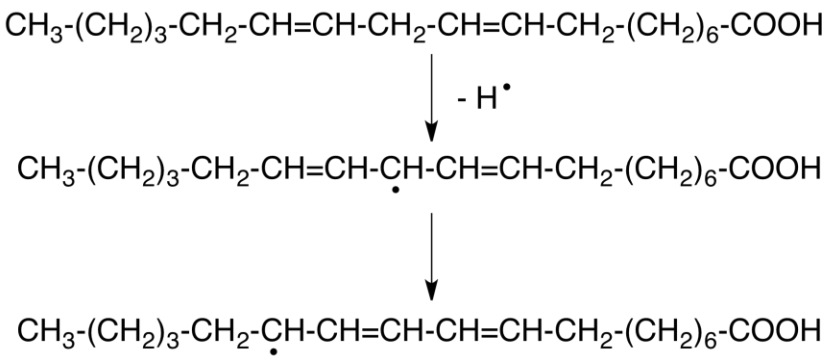


Figure 22. Formation of conjugated dienes following H abstraction from C11 in linoleic acid.

Patterns of conjugated diene development in stripped, fresh and steady-state oil during 3 hours heating at 180°C are shown in Figure 23. Similar patterns in the development of these degradation products appeared in all three oils, although the levels varied – steady-state > stripped > fresh. Conjugated dienes increased steadily throughout the heating period, then production slowed near the end of heating, especially in oils with higher pre-oxidation level. This apparent abatement in conjugated diene accumulation most likely results from increased rates of conversion of initial hydroperoxides to secondary structures without conjugated double bonds, particularly in steady state oils where some oxidation products were already present as catalysts.

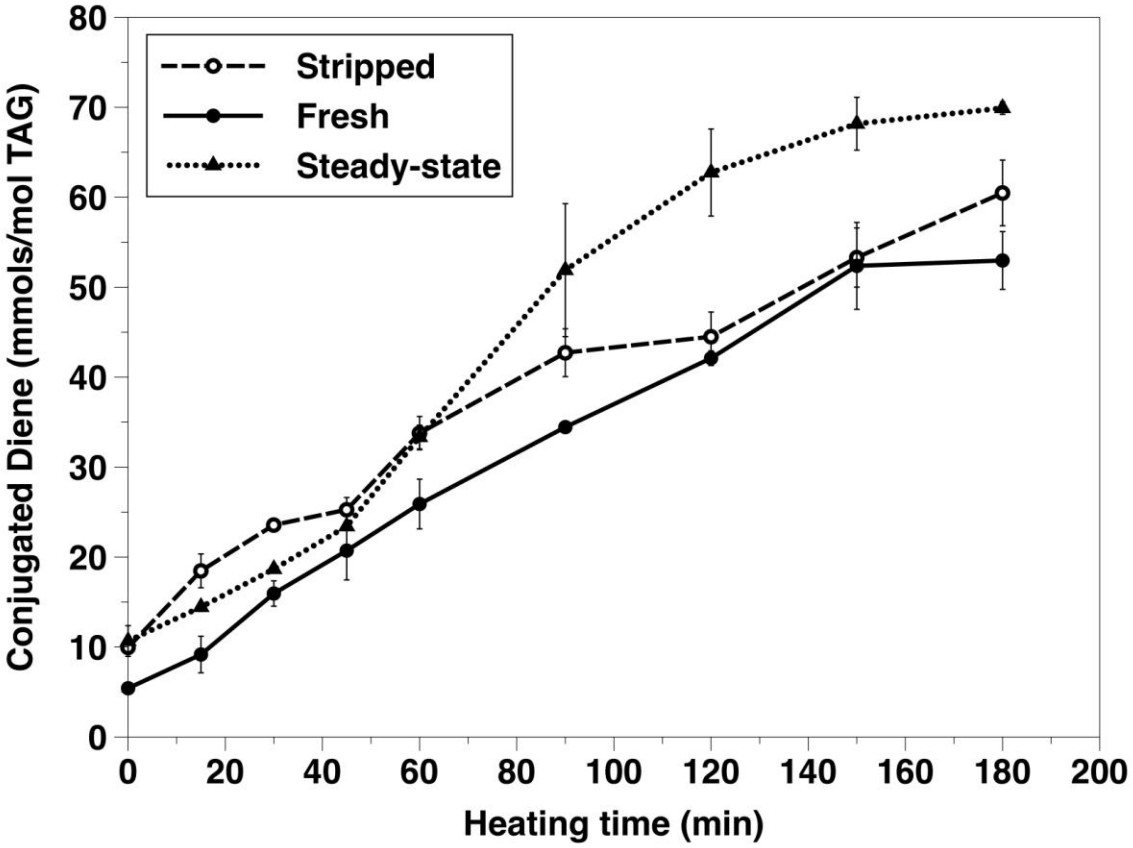


Figure 23. Conjugated diene development over 3 hours heating at 180°C under 2 bars air.

6.1.2 Hydroperoxides

Peroxide values are the most commonly used measure of lipid oxidation. Due to low stability, however, hydroperoxides are only suitable for determining extent of oxidation in early stages of oxidation where formation exceeds decomposition and peroxides are a direct measure of oxidation. However, as oxidation progresses and peroxide concentrations increase, or in the presence of light, metals, or heat, decomposition rates become faster than formation, and decomposition of hydroperoxides makes oxidation appear as though it was slowing when in fact it is accelerating in branching reactions. This is especially the case at high temperatures. For example, one

study conducted at room temperature and moderately elevated temperature (e.g. 60 °C) followed peroxide value for days without observing a breakdown of hydroperoxides [83]. In our research, however, hydroperoxides entered the net break-down phase (decomposition exceeding formation) within 45 minutes of heating (Figure 24). When oil was heated at 180°C (starting from 25°C), hydroperoxides built up fast, reached a peak (of different values at different time points depending on the oil), and then continued decomposing as long as heat was applied. The fact that high variations were observed despite numerous repeats of heating experiments demonstrates the instability of hydroperoxides. In fact, Nawar [9] had pointed out that peroxide tests are extremely sensitive to handling and especially temperature.

Stripped oil reached the highest peroxide levels in the shortest time (20-30 minutes) because metals and secondary oxidation products that catalyze hydroperoxide decomposition had been largely removed from the oil. Peroxides peaked at progressively lower concentrations and later times in fresh and steady-state oils, respectively. This

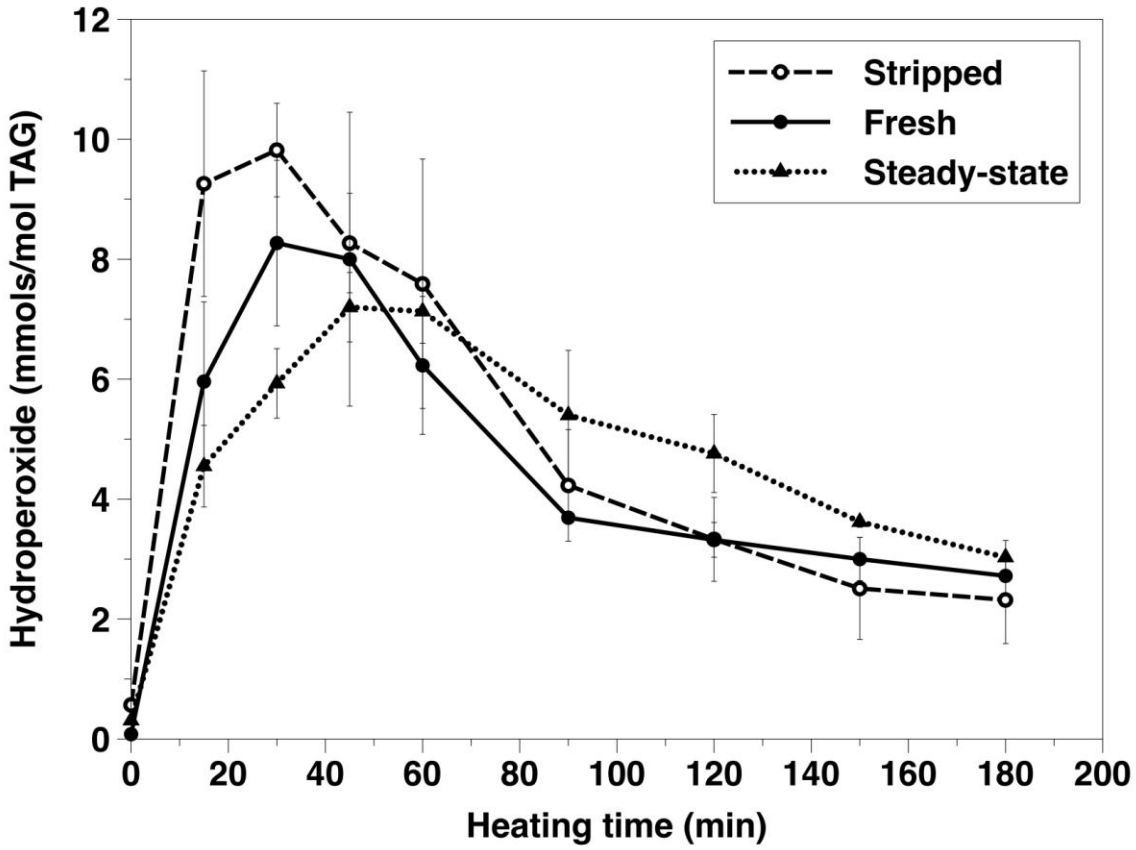


Figure 24. Hydroperoxide development over 3 hours heating at 180°C under 2 bars air.

shift occurred because components present in the oils facilitated decomposition and impeded accumulation of hydroperoxides. Steady-state oil, which had been previously heated and contained a variety of oxidation products as well as [probably] metals, showed the greatest interference with hydroperoxide accumulation during early heating, but retained higher peroxide levels at the end of heating. Note that in this case, lower peroxide levels alone cannot be interpreted as lower degradation or oxidation in the oil. Analyses of additional secondary products are necessary to distinguish the meaning of lower peroxides – rapid decomposition to other products, or true inhibited oxidation.

6.1.3 Development of aldehydes

During normal oxidation of lipids, aldehydes are a major class of compounds derived from decomposition of hydroperoxides [9]. Measurement of aldehydes is particularly important in frying studies since these products are major contributors of fried flavors. At ambient and moderately elevated temperatures, aldehydes do not accumulate until late stages of oxidation, and even then remain at levels substantially lower than hydroperoxides. In contrast, aldehydes were the major products observed at frying temperatures (Figure 25). The kinetics of aldehyde formation were particularly interesting: instead of forming after hydroperoxides had decomposed (the normal pattern in autoxidation), aldehydes began accumulating as soon as heating began, ahead of hydroperoxides, and they increased rapidly to a level far exceeding that of hydroperoxides in all three oils. This pattern will be shown even more clearly in Figures 28-30 in section 6.1.5. Peak hydroperoxide concentrations were 7-10 mmol/mol TAG while peak aldehyde concentrations were 100 to 150 mmol/mol TAG. Since starting values of hydroperoxides in these oils was very low, this pattern suggests that early aldehydes generated during heating derive from reactions other than decomposition of preformed hydroperoxides followed by scissions of the alkoxyl radicals. Similarly, it is difficult to account for the greater than order of magnitude excess of aldehydes over hydroperoxides and the speed of aldehyde formation simply by decomposition of hydroperoxides formed in accelerated autoxidation reactions, i.e. at C9 and C13 of linoleic acid. Thus, there must be additional reactions that occur before or parallel to hydroperoxide formation and decomposition that also generate aldehydes at a faster rate. The homologous series of short-chain aldehydes found in the vented headspace volatiles

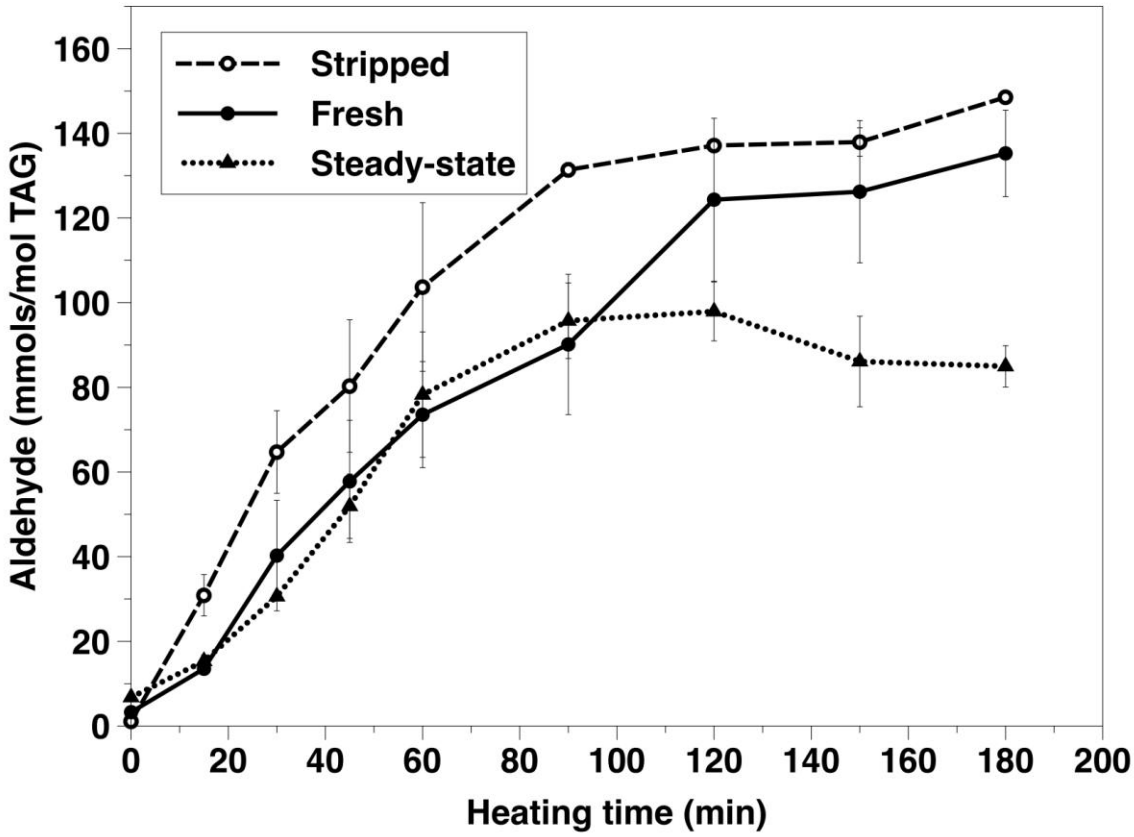


Figure 25. Non-volatile aldehyde development in oil over 3 hours heating at 180°C under 2 bars air.

(Figure 26) [79]-- a pattern not typical of oleic or linoleic acid autoxidation products -- supports this conclusion.

Two possible alternate sources of aldehydes not involving hydroperoxide intermediates are:

a) Thermal decomposition of O_2 to 2 $O^{\bullet}$ in the hot headspace, followed by direct addition of $O^{\bullet}$ to alkyl radicals, $R^{\bullet}$.

b) Local recombination of peroxyl radicals formed at thermal scission points to form tetroxides, followed by decomposition to alkoxyl radicals that transform to aldehydes

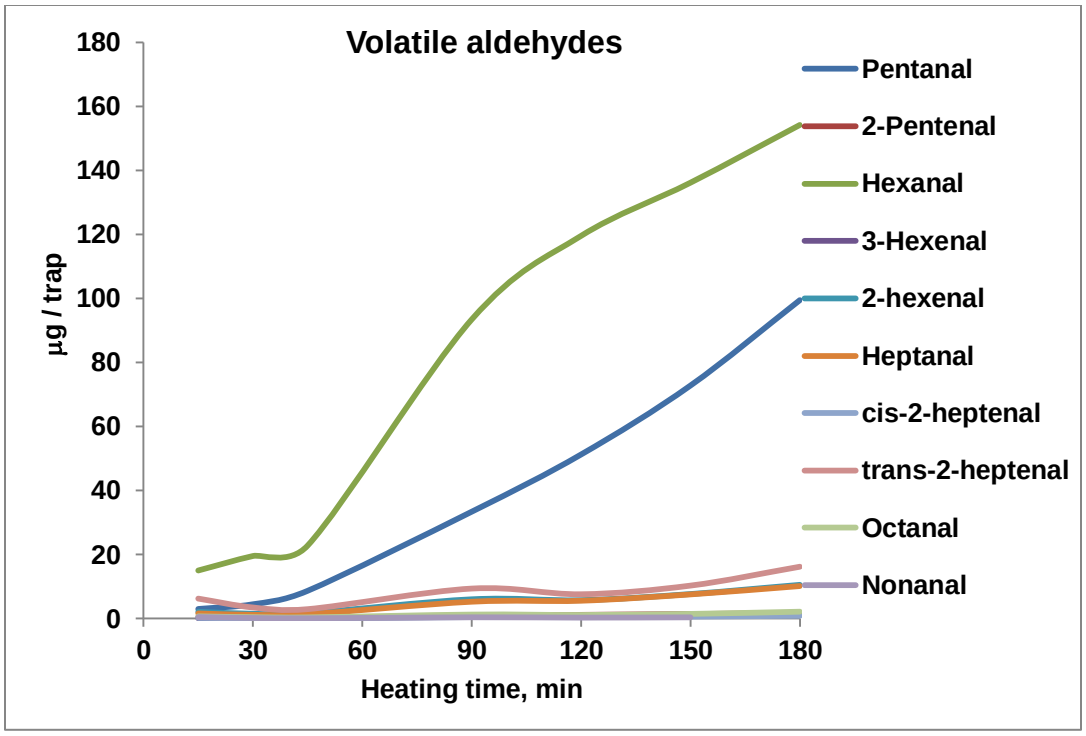


Figure 26. Non-volatile aldehyde development in oil over 3 hours heating at 180°C under 2 bars air. From Repko [79].

by the Russell mechanism [78]:

$$R_1OO^{\bullet} + {}^{\bullet}OOR_2 \rightarrow R_1\text{-}OOOO\text{-}R_2 \rightarrow O_2 + R_1O^{\bullet} + R_2O^{\bullet} \rightarrow R_1CHO + R_2CHO$$

The aldehydes detected in headspaces vented from heated oils are consistent with b) where the R groups are short chain fragments released after thermal scission and the peroxyl radicals are terminal rather than mid-chain.

Accumulation of aldehydes slowed down after 90 minutes in stripped oil and steady-state oil, and after 120 minutes in fresh oil. Such abatement logically occurs when aldehydes undergoes further oxidation and decomposition, and this is especially likely at high temperatures. For example, saturated aldehydes can oxidize to corresponding acids, or participate in dimerization and condensation reactions; unsaturated aldehydes can

undergo condensation reactions as well as further autoxidation, giving rise to polymers, short-chain hydrocarbons, aldehydes, and dialdehydes [9]. Slowed production of aldehydes may also occur if oxygen becomes limited.

6.1.4 Free fatty acids

Patterns of free fatty acid development in the three oils are shown in Figure 27. Free fatty acids did not form immediately after heating began, but started to accumulate after 30, 60, and 45 minutes in stripped, fresh and steady-state oils respectively. Furthermore, as will be shown in a later section, oxygen is required for acid formation, and no acids were detected when oils were heated under inert gases. These results are completely inconsistent with the standard explanation of frying chemistry that cites ester bond hydrolysis as the first chemical change in heated oils. Oxidation of aldehydes to corresponding fatty acids is a very facile reaction that can proceed even without catalysts [84, 85]. Under normal conditions, the oxygen is supplied by water:

$$RCHO + H_2O \rightarrow RCOOH + 2H^+ + 2e^-$$

In the neat heated oils, the oxygen can be supplied from the headspace or decomposition of hydroperoxides. The oxygen dependence of fatty acid formation plus the homologous series of short-chain fatty acids detected in the headspace along with short-chain aldehydes of the same chain lengths lead us to propose that the majority of fatty acids in low moisture systems are derived from oxidation of aldehydes rather than ester hydrolysis, at least in early stages of thermal degradation. Effect of O_2 on fatty acid concentration, as well as auxiliary tests to verify this hypothesis will be discussed in the next section.

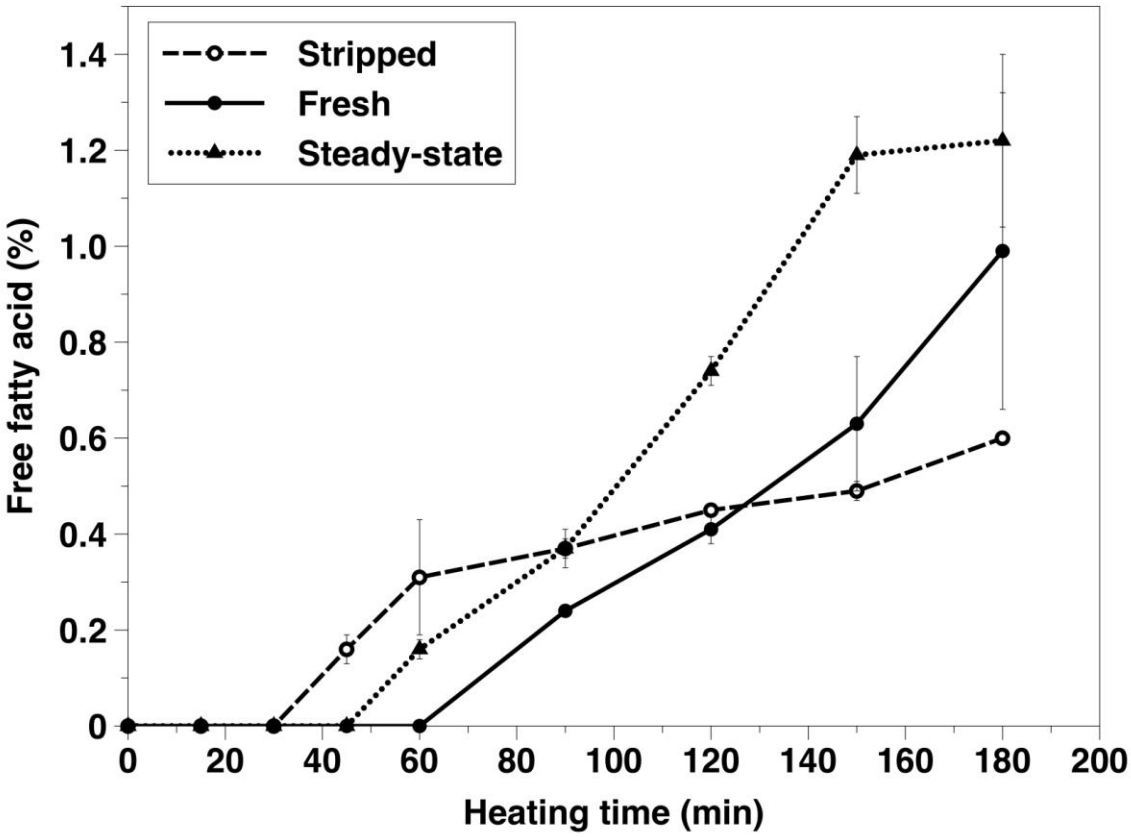


Figure 27. Pattern of free fatty acid development over 3 hours heating at 180°C under 2 bars air.

6.1.5 Relationships between products

In order to determine relationships between oxidation products in terms of magnitude and kinetics, the four degradation products were plotted in the same graph for each oil. Figures 28, 29, 30 show patterns of conjugated dienes, hydroperoxides, aldehydes and free fatty acids formed in stripped oil, fresh oil and steady-state oil, respectively. These plots make it very clear that aldehydes were the dominant oxidation product of those measured; they appeared to be the first formed, and their concentrations were order an of magnitude higher than conjugated dienes, hydroperoxides, or fatty acids

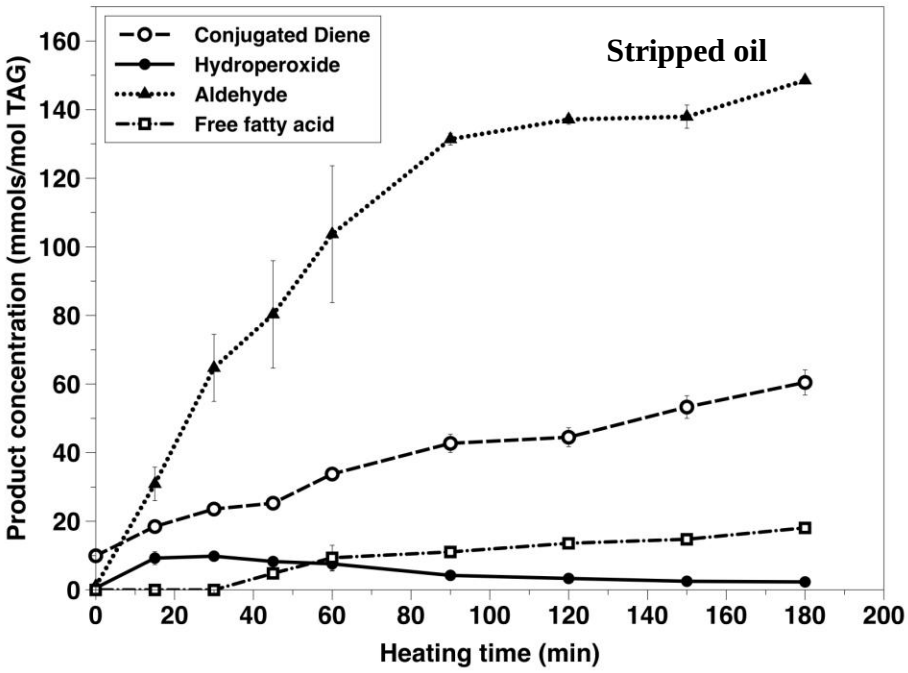


Figure 28. Relative kinetics of formation for major thermal degradation products in stripped oil over 3 hours heating at 180°C under 2 bars air.

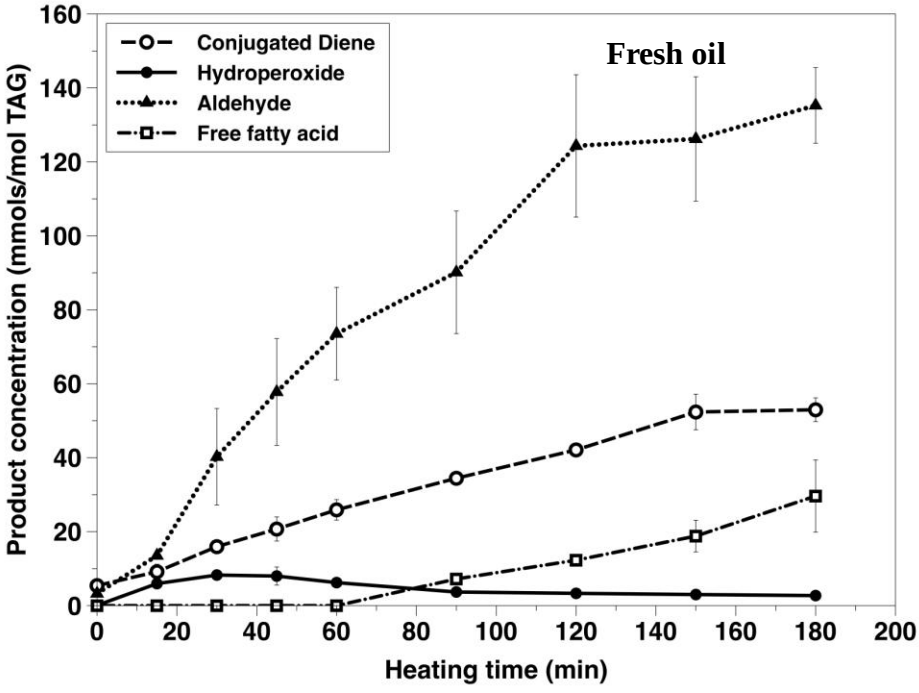


Figure 29. Relative kinetics of formation for major thermal degradation products in fresh oil over 3 hours heating at 180°C under 2 bars air.

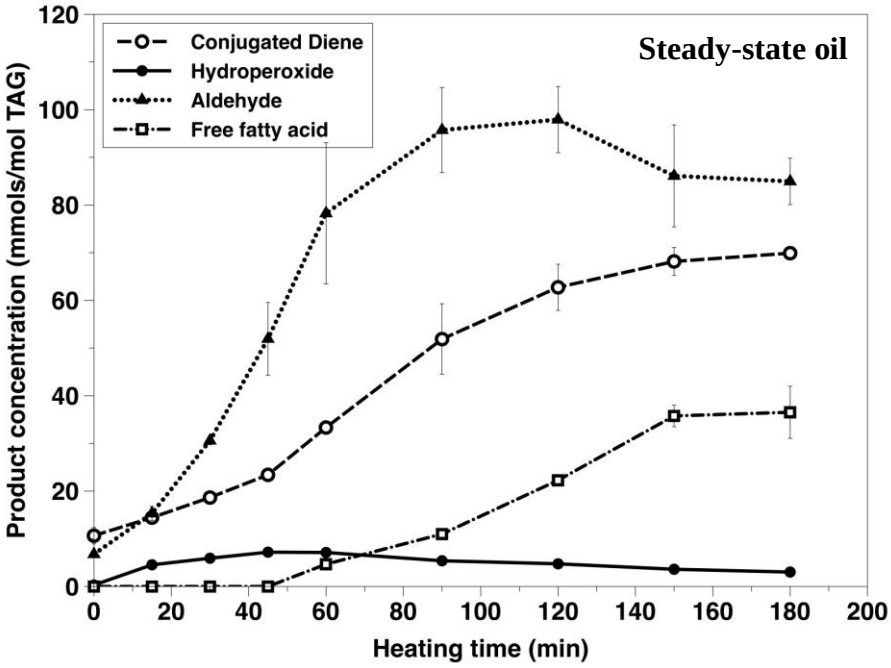


Figure 30. Relative kinetics of formation for major thermal degradation products in steady-state oil over 3 hours heating at 180°C under 2 bars air.

in some oils. This pattern is in distinct contrast to autoxidation, where aldehydes are late products and remain at low concentrations relative to hydroperoxides (Figure 18). In this study with oil heated at 180°C, the fact that aldehydes formed faster and at so much higher concentrations of even conjugated dienes, is evidence that at least some aldehydes are formed by pathways not involving autoxidation reactions as precursors. At this high temperature when all chemical reactions are greatly accelerated, there might be an extensive level of LOOH formation and decomposition "behind the scenes", generating alkoxyl radicals to form aldehydes. However, such a process is slow while aldehydes in this study were generated very rapidly.

The relative product kinetics raise some additional interesting questions about mechanisms. In autoxidation, conjugated dienes commonly form parallel to or just

slightly ahead of hydroperoxides. However, at frying temperatures, conjugated dienes remained at much higher levels that hydroperoxides and increased throughout the heating period. Low hydroperoxides could be explained by thermal decomposition, but then conjugated dienes would be lost in subsequent degradations to secondary products. This did not happen, so there are some disconnects between hydroperoxides and conjugated dienes. One alternative explanation is that the hydroperoxides being detected are primarily terminal hydroperoxides formed at thermal scission points, and decomposition of these hydroperoxides generates radicals that abstract hydrogens from neighboring acyl chains. The product of this reaction is a conjugated diene that may or may not add oxygen and subsequently form hydroperoxides. Furthermore, there may be secondary pathways that do not involve hydroperoxide formation and decomposition but generate radicals in conjugated double bond systems. These pathways either form stable products that retain the conjugated diene structure, or regenerate conjugated dienes at a rate much faster than transformations to any secondary products.

That fatty acids begin to accumulate as aldehyde production slows is consistent with oxidation of aldehydes to carboxylic acids.

Oxidative degradation was tremendously accelerated and enhanced in the steady-state oil compared to stripped and fresh oils. Aldehydes were produced most rapidly in this pre-oxidized oil but reached lower final levels. In contrast, levels of conjugated dienes and carboxylic acids were notably increased. This suggests that some components of the steady-state oil divert conversion of thermal scission products from aldehyde formation to hydrogen abstraction, and also induce more rapid oxidation of the aldehydes

to acids. This is still one more piece of evidence that there are reactions important in thermal degradations that have not yet been identified.

6.2 FACTORS AFFECTING DEGRADATION

6.2.1 Effects of oil pre-oxidation

Analyses of pre-oxidation levels of stripped, fresh and steady-state oil provided by the supplier are shown in Table 3. The expectation in testing these oils was that if pre-formed oxidation products catalyzed degradation during heating, the steady-state oil would show the highest oxygen consumption and product levels and stripped oil with oxidation products removed would show the least. This pattern was indeed observed in oxygen consumption where oxygen consumption rates and total oxygen consumption both increased in the order stripped < fresh < steady-state (Table 4); the difference between the three oils was significant ($p < 0.05$).

Table 3. Characterization of stripped, fresh and steady-state oils (information provided by supplier) (PV: Peroxide value; p-AV: p-anisidine value, index of 2-alkenal concentration in the oil; FFA: Free fatty acid).

	Stripped	**Fresh**	**Steady-state**
PV, meq/kg	0.43	3.13	0.31
p-AV, ppm	0.38	2.90	3.00
FFA, wt%	0.03	0.01	0.02
Tocopherols, ppm	194	723	813
Moisture, wt%	0.41	0.01	0

Table 4. Oxygen consumption rate and total oxygen consumption of stripped, fresh and steady-state oil.

	Max rate (mmols O_2/min)	**O_2 consumption (mmols O_2/mol TAG)**
Stripped	0.010±0.000	86.87±1.95
Fresh	0.012±0.000	98.04±1.97
Steady-state	0.019±0.001	130.56±1.97

Oxidation products generally followed the same pattern but not completely. Steady-state oil produced the highest levels of conjugated dienes, hydroperoxides, and free acids, but had the lowest proportional increase in products (Table 5). Stripped oil had lower primary products but much larger amounts of aldehydes. Stripped oil also degraded more than fresh oil, probably due to reduction of protective tocopherols and citric acid. Tocopherol acts as an antioxidant in two mechanisms: the phenol form competes with unsaturated fatty acid for peroxyl radicals (LOO$^{\bullet}$) to slow propagation and the formation of new alkyl radicals (L$^{\bullet}$), and the tocopheryl quinone and semiquinone react with L$^{\bullet}$ to regenerate LH (Figure 31) to prevent the chain reaction from starting [86]. Low tocopherol levels thus allowed enhanced formation of both conjugated dienes and aldehydes in stripped oil. Low hydroperoxides and high aldehydes may also be due to higher metal contamination that occurred in the stripped oil, even though the stripping process should have removed them.

Table 5. Concentration of degradation products in oils before starting and after 3 hours heating. (CD: Conjugated diene, LOOH: hydroperoxide. Aldehyde, all in mmols/mol TAG; FFA: Free fatty acid, in % oil weight).

	Stripped		**Fresh**		**Steady-state**	
	0 min	180 min	0 min	180 min	0 min	180 min
CD	9.96±0.00	60.48±3.65	5.42±0.06	52.97±3.21	10.67±1.71	69.91±0.69
PV	0.57±0.01	2.32±0.73	0.08±0.04	2.72±0.09	0.31±0.06	3.03±0.28
Aldehyde	1.12±0.00	148.53±1.07	3.27±0.17	135.27±10.21	6.78±0.20	84.96±4.88
FFA	ND	0.60±0.01	ND	0.99±0.33	ND	1.22±0.18

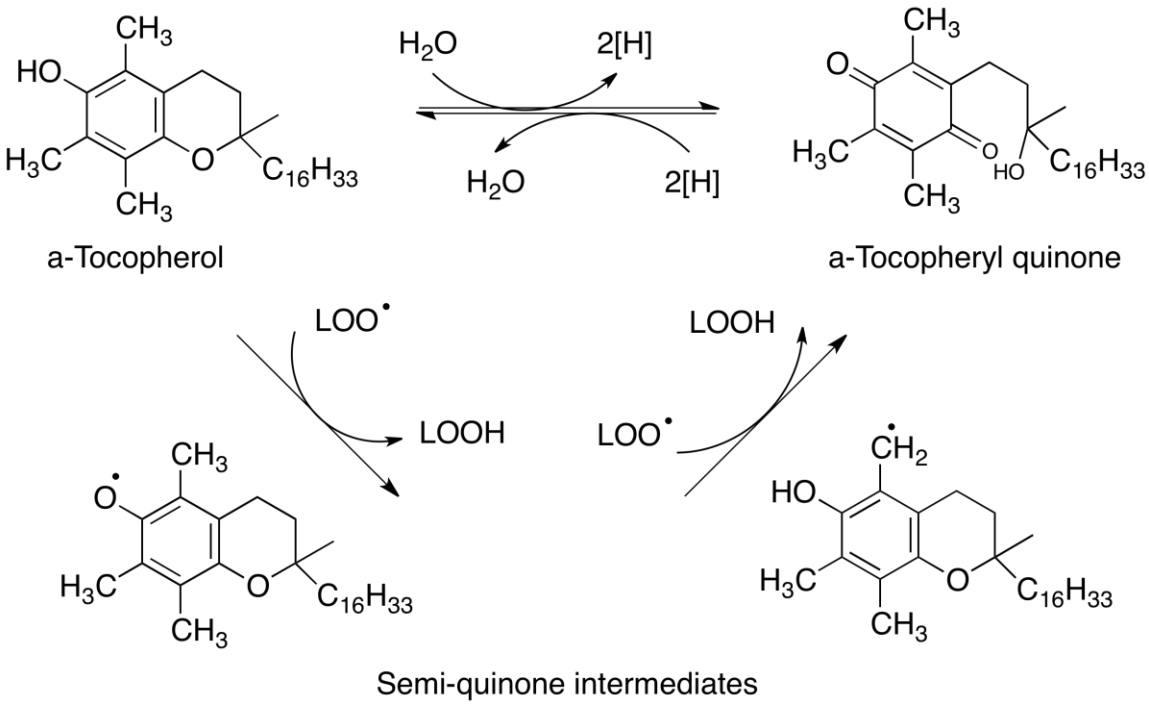


Figure 31. Reactions involved in tocopherol interference with lipid oxidation. Adapted from Cuppett [86].

When assessing effects of pre-oxidation on thermal degradation, we must also consider the generation kinetics for each of the measured oxidation products that were presented in Figures 23-25 and 27 in the previous section. Formation of conjugated dienes occurred at comparable relatively constant rates in stripped and fresh oils (Figure 23) indicating similar radical generating process and the innate susceptibility to hydrogen abstraction. Steady-state oil began heating in line with the other oils, but rates accelerated then plateaued as heating progressed, so either radical generating process was different or the oil's susceptibility to oxidation was altered. Either explanation is possible considering that the additional oxidation products present in the starting oil should be more sensitive to thermal decomposition and also should form oxygenated radicals that are stronger hydrogen abstractors than the peroxyl radicals formed after initial thermal scissions. At the same time, the slower accumulation and plateaus of conjugated dienes and aldehydes and the accelerated accumulation of acids certainly suggest that in the matrix already containing mixed oxidation products, secondary reactions of radicals and break down of intermediates are greatly enhanced, and the system moves more rapidly to advanced degradation stages via pathways and products not analyzed in this study.

Fresh oil showed the greatest increase (10-fold) in conjugated dienes, while the stripped and steady-state oils increased by about six times. This and the other behaviors described above clearly indicate that different underlying reactions were in play in the three oils.

6.2.2 Effects of oxygen

Effects of oxygen on frying oil degradation were evaluated by heating steady-state oil in Oxipres cells pressurized with 2 bars of gases containing decreasing concentrations of oxygen: air (21% O_2), 5% O_2, 2% O_2, and N_2. An additional test was conducted pre-sparging the oil with argon to displace/eliminate dissolved oxygen in the oils, then heating under N_2.

Figure 32 shows the oxygen consumption curves over the full 3 hours of heating. Times to reach peak pressure and peak pressure values are listed in Table 6. At 180 °C, no induction period was present for any treatment, even when the heating was conducted under extremely low pO_2. Preliminary tests found that induction periods present at 100°C and 120°C disappeared when temperatures were raised above 150°C. This suggests a major shift in mechanism. Results are consistent with a process in which thermal scission begins immediately upon heating and leads to products whose formation depends on oxygen availability. Under conditions of no or low oxygen, radicals formed by thermal scission have three possible fates: recombine initial fragments to reform the parent compound, recombine disparate fragments to form new compounds and dimers, or anneal to form fragment products. When sufficient oxygen is present, oxygen adds to thermal scission radicals to form terminal peroxyl radicals that abstract hydrogens, generate hydroperoxides, and initiate secondary radical chain reactions, or, alternatively, transform to other products independently of hydroperoxides.

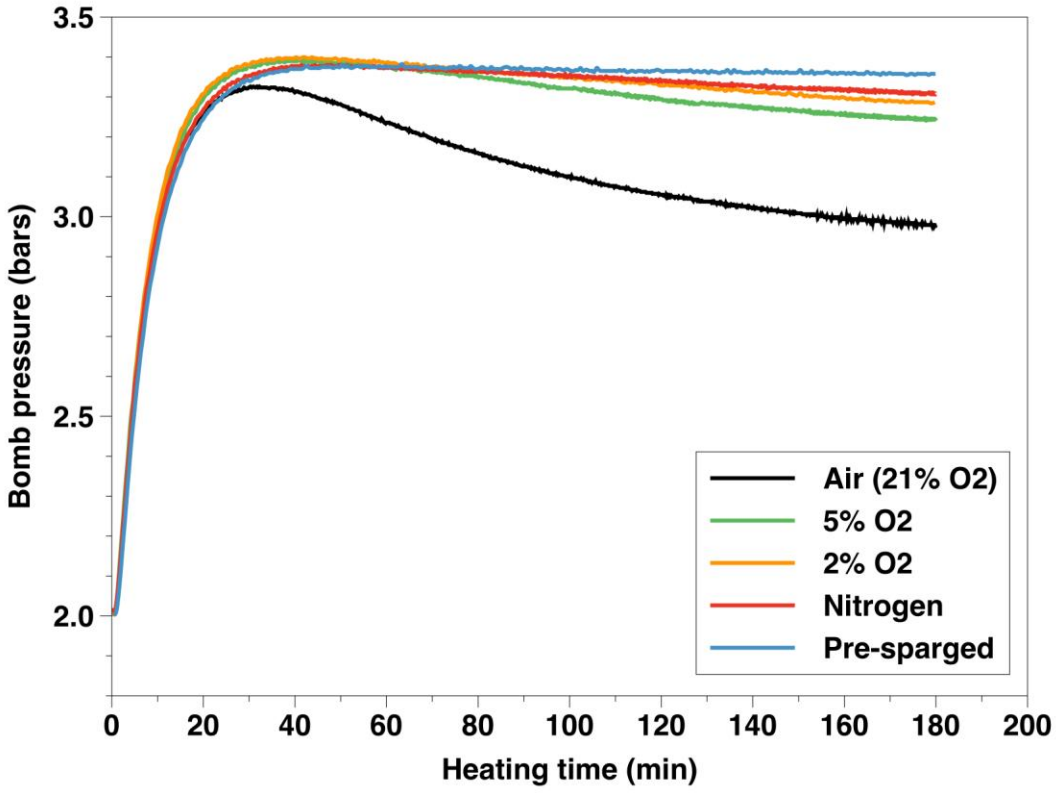


Figure 32. Oxygen consumption curve of steady-state oil heated at 180°C in gases of different oxygen concentration in the headspace and in the oil.

Table 4. Time to peak and peak value of steady-state oil oxygen consumption curve heated in different gases.

	Peak	
	min	**bars**
Air	31.65±1.93	3.34±0.01
5% O_2	39.02±2.29	3.38±0.02
2% O_2	40.91±0.93	3.39±0.01
N_2	45.40±5.73	3.38±0.01
Sparged	61.68±2.44	3.37±0.01

Not much difference was observed between peak oxygen pressures, though the time to reach peak pressure increased as [O_2] decreased in the different gases, in the order of air < 5% O_2 <2% O_2 < N_2 < Sparged. This difference reflected the competition for oxygen and reduction in cell pressure that began immediately with heating, with more oxygen, radicals became oxygenated faster as they were formed and the point at which oxygen consumption exceeded pressure increase due to temperature occurred earlier. It is strictly an equilibrium process.

It was strongly evident in Figure 32 that rate of oil oxidation increased with the percentage of oxygen present in the headspace. To compare oxygen consumption rates more clearly, unconfounded by differences in peak pressures, all curves were normalized to the same peak value, i.e. assuming all samples started to decompose from the same pressure from the same time (set as time zero) (Figure 33). Maximum oxygen consumption rates and total oxygen consumption were calculated from these curves (Table 7). Oxygen consumption rates and total consumption decreased as [O_2] decreased, and the differences were all significant ($p<0.05$) when compared pairwise, except for 5% versus 2% O_2. Nitrogen gas, even when pre-purified, has some oxygen contamination, and this probably accounts for the low level of oxygen consumption observed when cells were pressurized with nitrogen. Pre-sparging oil with argon nearly shut down oxidation. Results of this experiment indicate that 1) it is difficult to deplete all oxygen in the system even with inert gas in the headspace or when oil is sparged with inert gas, and 2) trace levels of oxygen are sufficient to fuel oxidation during thermal degradation.

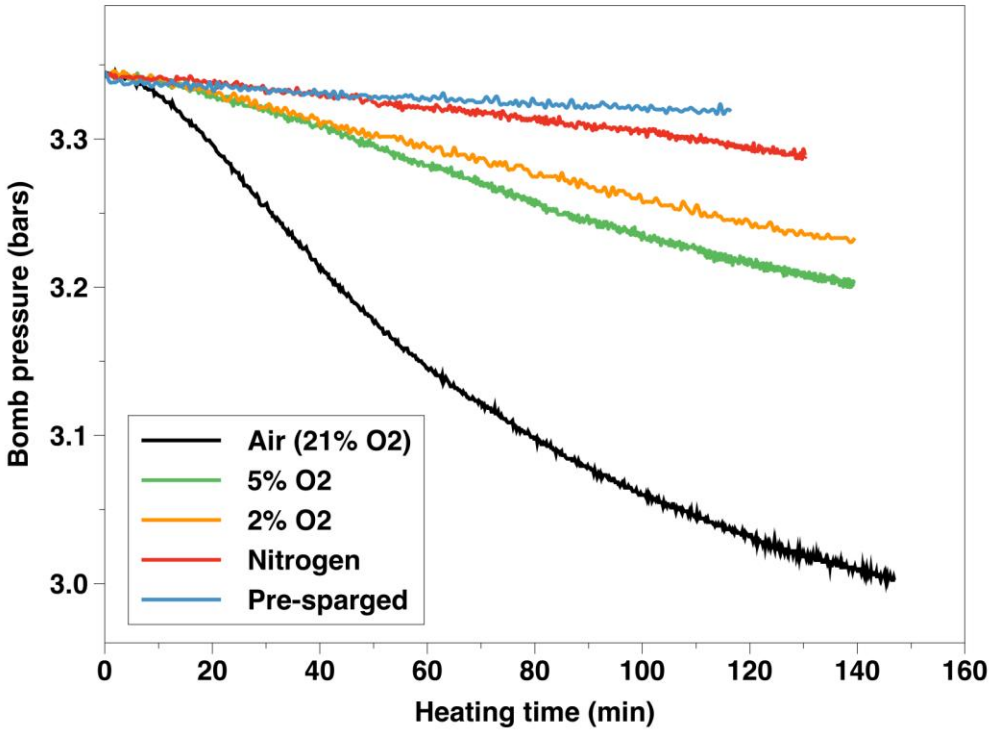


Figure 33. Normalized oxygen consumption curve of steady-state oil heated at 180°C in gases of different oxygen concentration in the headspace and in the oil.

Table 5. Oxygen consumption rate and total oxygen consumption of steady-state oil without pre-sparging heated in air, 5% O_2, 2% O_2, N_2 and with argon sparging heated in N_2.

	Max rate (mmols O_2/min)	**O_2 consumption (mmols O_2/mol TAG)**
Air	0.019 ± 0.001	103.56 ± 1.97
5% O_2	0.009 ± 0.000	54.83 ± 3.94
2% O_2	0.005 ± 0.000	44.17 ± 2.01
N_2	0.007 ± 0.000	25.09 ± 3.94
Sparged	0.002 ± 0.000	10.22 ± 1.31

Analysis of degradation products throughout the course of heating provided strong evidence for dramatic effects of oxygen on frying oil degradation (Figure 34 and Table 8). Indeed, of all factors tested to date, oxygen is by far the major controlling factor. The air curve (blue) was dramatically higher than curves of reduced oxygen samples for all products. LOO$^{\bullet}$ and LO$^{\bullet}$ radicals formed in air abstract hydrogen from allylic carbons in polyunsaturated fatty acids, resulting in formation of conjugated dienes and hydroperoxides; suppression of oxy radical formation by reduced oxygen atmospheres decreased formation of conjugated dienes (Figure 34a). In air, hydroperoxides formed rapidly followed by extensive decomposition, but when [O_2] was reduced, [LOOH] correspondingly decreased and decomposition was effectively inhibited (Figure 34b). Notably, LOOH, aldehydes, and free fatty acids were nearly shut down when O_2 was excluded more rigorously in Ar-sparged oils. This demonstrates the effectiveness of oxygen limitation in stabilizing frying oils and extending their useful fry life.

The most interesting observation was the development of free fatty acids (Figure 34c), not only in their delay in formation, even after the oil had reached temperature, but also that the extent of this delay as well as final fatty acid concentrations were totally controlled by [O_2]. These results indicate that in dry oils 1) release of free fatty acids from triacylglycerols is not the first chemical degradation as almost universally described in the literature, and 2) hydrolysis, which should be oxygen-independent, is not the major source of fatty acids detected in this system.

Volatile short-chain carboxylic acids of the same chain length as aldehydes were observed in volatiles trapped from these oils, and increased with heating time and oxygen

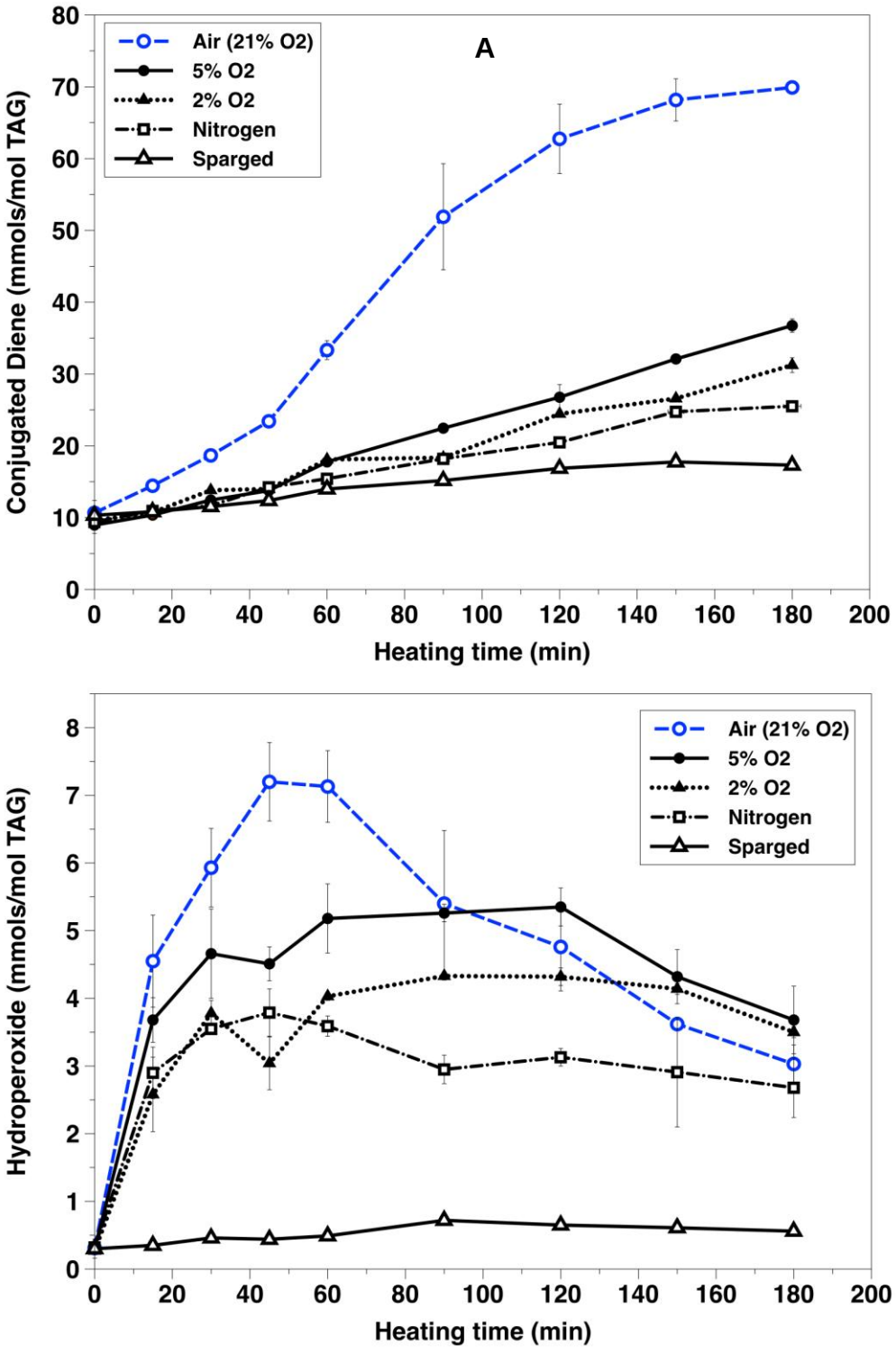
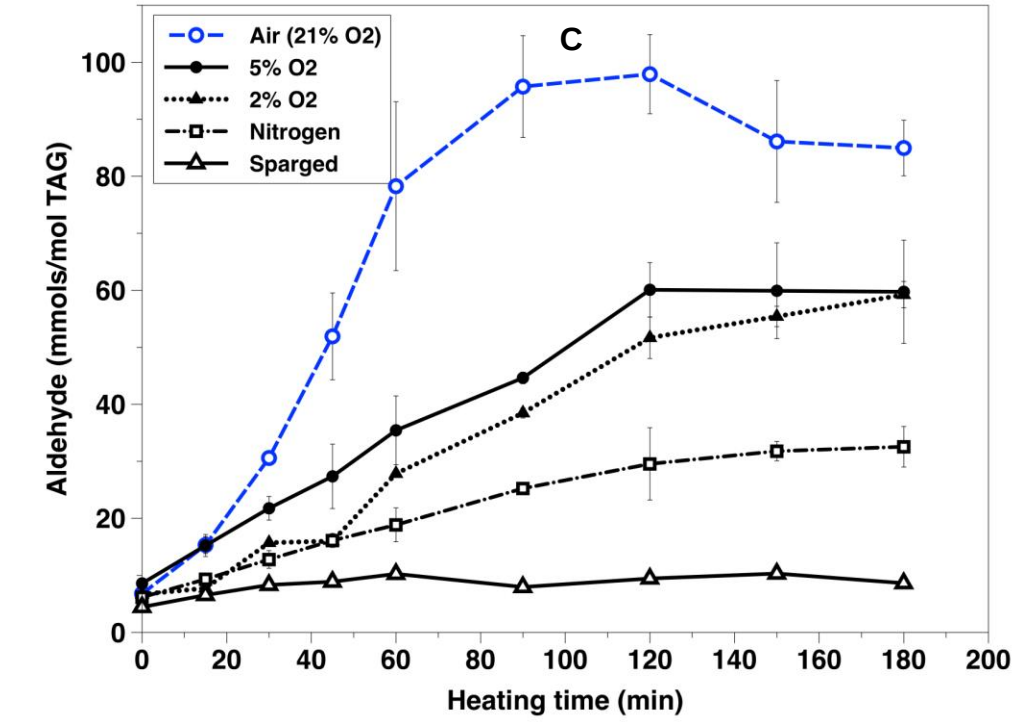


Figure 34. Development of conjugated dienes (A) and Hydroperoxides (B) in steady-state oil over 3 hours heating in different [O_2] environments



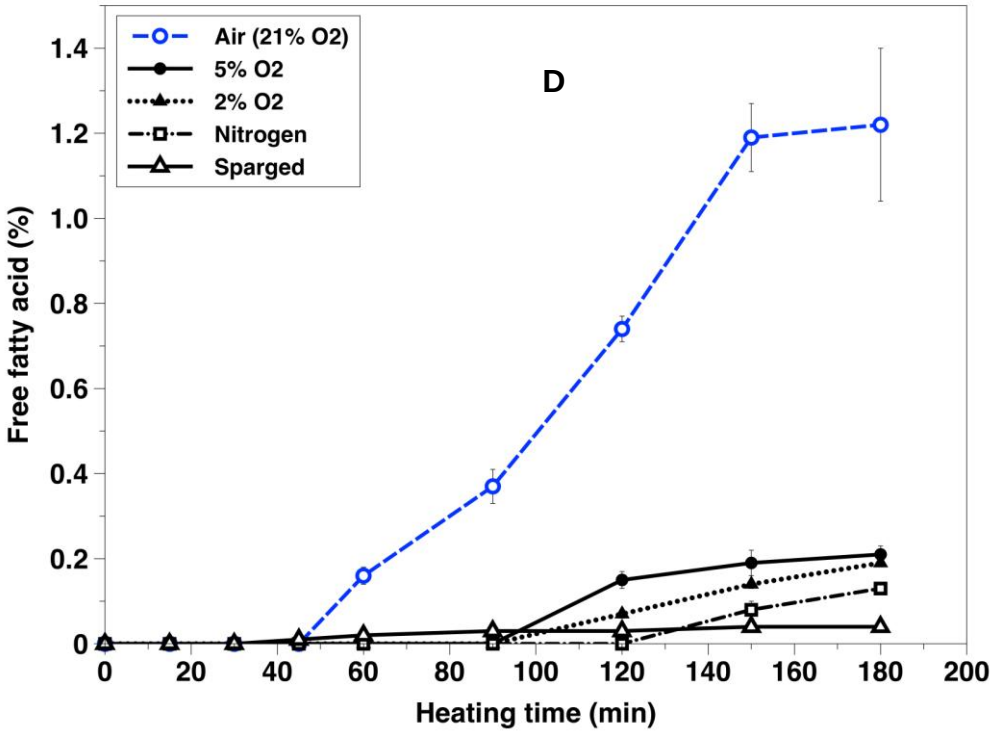


Figure 34 continued. Development of aldehydes (C) and free fatty acids (D) in steady-state oil over 3 hours heating in different [O_2] environment.

Table 6. Levels of degradation products formed in steady-state oil after heating under different oxygen levels.

	Conjugated diene (mmols/mol TAG)	Hydroperoxide (mmols/mol TAG)	Aldehyde (mmols/mol TAG)	Free fatty acid (%)
Air	69.91±0.69	3.03±0.28	84.96±4.88	1.22±0.18
5% O_2	36.75±0.94	3.68±0.50	59.74±9.06	0.21±0.02
2% O_2	31.24±1.03	3.50±0.08	59.23±2.31	0.19±0.00
N_2	25.52±2.24	2.68±0.44	32.56±3.56	0.13±0.00
Sparged	17.31±0.33	0.56±0.02	8.63±0.13	0.04±0.00

levels [79]. These observations suggested that carboxylic acids can be produced by oxidation of aldehydes formed from thermal scissions. To test this possibility, 1000, 2000, and 5000 ppm hexanal were added to stripped oil that was heated for 3 hours under 2 bars air. Interestingly, free fatty acid levels increased linearly although not quantitatively with added hexanal (R^2=0.9949). FFAs detected exceeded that of hexanal added by about 10 times (Figure 35). At the same time, there was a linear increase in headspace hexanal, irregular increase in hexanoic acid, and increase in other hexanal decomposition products: hexanone and pentanone isomers doubled, and pentyl and butyl formate esters tripled (Table 10) showing that hexanal (and presumably other aldehydes) does indeed oxidize to carboxylic acids but also degrades competitively by other pathways at the same time. Decreases in some volatile aldehydes would tend to support the possibility that the hexanal catalyzed oxidation of other aldehydes [79]. However, the experiment needs to

be repeated with hexanal heated alone and in oils with identification of the individual fatty acids formed in order to decipher reactions leading to this remarkable increase in free fatty acid concentrations.

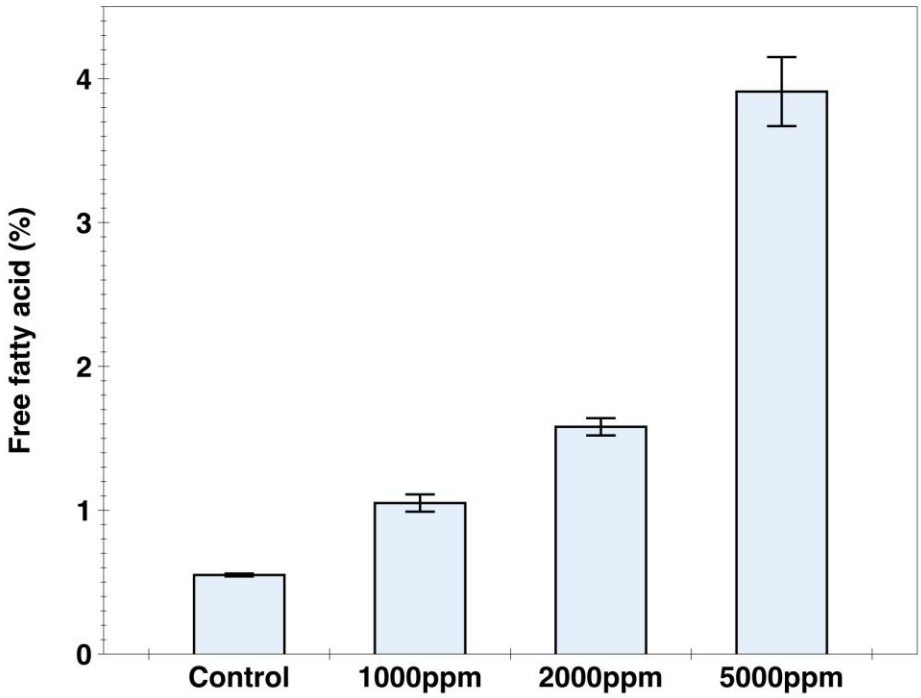


Figure 35**.** Increase in free fatty acid levels in oils with 1000, 2000 and 5000 ppm hexanal added before heating 3 hours at 180 °C.

Table 7. Volatiles released from oils with hexanal added before heating 3 hours at 180 °C.

	0	1000 ppm	2000 ppm	5000 ppm
Pentane/Isopentane	204.56	336.90	374.02	283.59
Hexanal	106.98	127.56	162.68	213.26
Pentanal	96.16	94.41	90.05	82.39
2- Pentenal	2.47	2.77	2.33	1.70
2- Hexenal	20.37	15.99	8.27	14.22
cis-2-Heptenal	2.26	1.72	0.50	1.65
trans-2-Heptenal	46.04	27.13	8.93	26.38
Nonanal	2.80	0.81	0.88	1.18
Acetic acid	2.28	3.86	20.48	15.10
Pentanoic acid	2.32	2.67		3.02
Hexanoic acid	16.17	26.14	8.89	27.91
2-Pentanone	24.78	27.23	32.92	44.60
3- Hexanone?	2.70	3.20	3.09	4.18
2- Hexanone/ 1-Octene	6.83	7.16	9.78	8.27
Butyl Formate	2.45	3.53	7.54	9.12
Pentyl Formate	9.11	8.88	21.17	26.07

6.2.3 Effects of water

Water becomes an inevitable contaminant in frying oils when foods are added. Water by itself obviously catalyzes hydrolysis when acids or heat are present, but its effects are much more complex when several elements are present in the oil. For example, water may hydrate and stabilize free radicals, free fatty acids, phospholipids, hydroperoxides and metals [87-90], all protective effects. On the other side of the reactivity ledger, water provides microregions that may enhance electron transfer of metals and proton transfer from other compounds; it also accelerates decomposition of secondary oxidation products such as aldehydes [91] and alters distribution of secondary products [92]. While all these actions are recognized individually, effects and mechanisms of multiple molecular interactions involving water are not yet understood. Therefore, water effects on stability is an important perspective needing more detailed investigation, both individually and in combination with other factors. Interactions involving multiple factors will be discussed later.

In industrial potato chip frying, 2% is the average equilibrium moisture level remaining in steady-state oils after potato slices have been added and most of the fluid water has flashed off as water vapor (privileged communication). Therefore, we tested 2% (wt/wt) water level and a lower level of 0.5%.

To take the interference of water vapor to headspace pressure into consideration, a water control was run with every water added sample, in which the exact amount of water added into oil samples was deposited in reaction vial and heated under same conditions as oil blank control and treated samples. The average of O_2 consumption rate and amount by water blank control was listed in Table 8. All O_2 consumption data listed in later sections

that involves water was numbers after subtraction of water blank.

Table 8. O_2 consumption of water control blank of 0.05g (0.5%) and 0.2g (2%).

	Max rate (mmols O_2/min)	**O_2 consumption (mmols O_2/mol TAG)**
0.05g	0.001 ± 0.000	22.30 ± 3.68
0.2g	0.008 ± 0.001	81.77 ± 4.29

After subtraction of the water blank, both the rate of oxygen consumption and the total oxygen consumed decreased when water was present, and the decrease was more significant in the 2% water sample (Table 11). This decrease may well come from reduced access of oxygen to oil caused by a water vapor barrier forming over the oil surface as proposed by Dana et al (yr), but may also result from stabilization of hydroperoxides and annealing of thermal scission radicals so that oxygen addition and initiation of radical chains is limited.

Table 9. Changes in O_2 consumption rate and total O_2 consumption in steady-state oil heated 3 hours with 0.5% and 2% added water.

% water	**Max rate (mmols O_2/min)**	**O_2 consumption (mmols O_2/mol TAG)**
Control (0%)	0.020 ± 0.003	134.28 ± 1.97
0.5%	0.016 ± 0.000	117.09 ± 6.57
2.0%	0.015 ± 0.000	98.82 ± 9.86

However, effects of water are not straightforward because oxidation products did not consistently follow decreased oxygen consumption. Conjugated dienes increased

somewhat (<10%) in both 0.5% and 2% samples (Figure 36a), while hydroperoxides and aldehydes decreased at the end of 3 hours (Figures 36b and c, respectively). The aldehyde decrease in samples with 2% water was statistically significant ($p<0.05$) (Figure 36c).

Mosca et al. [93] reported accelerated oxidation in W/Olive oil emulsions versus olive oil itself in the presence of a radical initiator, and speculated that radical build up at the W/O interface might be responsible for higher rates of oxidation in emulsions. A similar increase in conjugated dienes in heated frying oils might be caused by activation of trace contaminant metals and enhanced electron or H abstraction from allylic carbons by water. This would increase conjugated dienes, but if oxygen access was limited, subsequent formation of hydroperoxides would correspondingly be depressed. Activation of metals by water should increase rates of hydroperoxide decomposition (observed) and also generation of secondary aldehydes. The latter was not observed because water enhanced transformation of aldehydes to secondary products [91] or because water caused a shift in product pathways so that other unmeasured products were preferentially formed in place of aldehydes. A shift in degradation pathways to epoxides and ketones is consistent with lower peroxides and aldehydes and also with lower oxygen consumption because these products have one rather than two O atoms added.

Surprisingly, not much change was observed in free fatty acids (Figure 36d), which again suggests that hydrolysis of triacylglycerols is a minor source of fatty acids under high temperatures. If water is an inducing factor, levels much higher than the average steady-state concentrations in industrial frying, e.g. of potato chips and snacks, must be required.

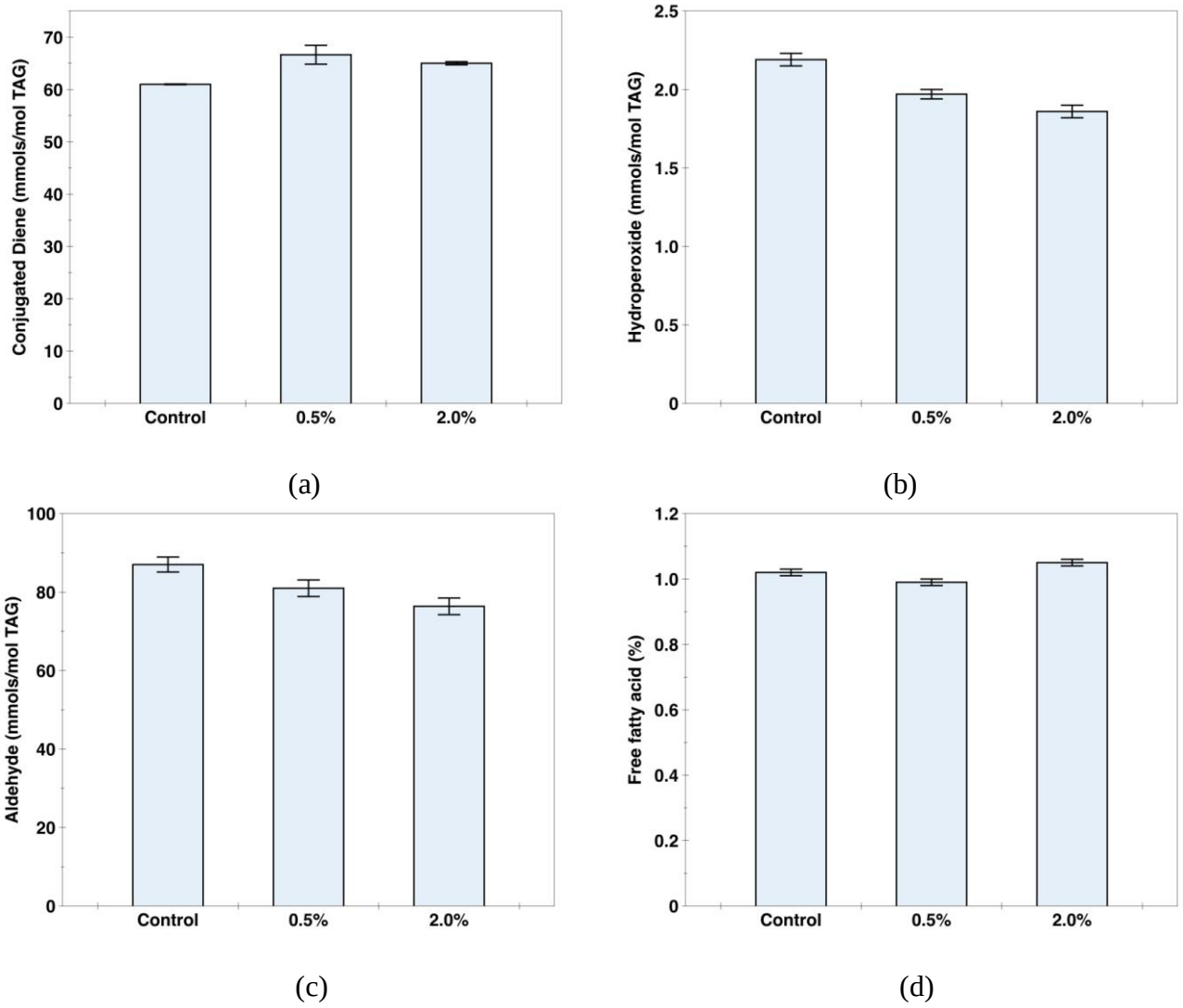


Figure 36. Levels of conjugated dienes (a), hydroperoxides (b), aldehydes (c), and free fatty acids (d) in oils heated three hours with 0.5% and 2% added water.

6.2.4 Effects of metals

Numerous studies have cited metal contamination, particularly iron, as a key factor limiting stability of frying oils and shelf life of foods fried in oils. At the same time, determining the role of metals in degradation of frying oils is not simple because metals behave differently in aprotic media and relatively little is yet known about oil-phase reactions of metals [94], metals are not soluble in oils so introducing them quantitatively is difficult, metal-mediated reactions are in competition with thermal processes for causing degradation, and metals can be antioxidants by converting radicals to ions as well as pro-oxidant by generating radicals [94]. Most early studies with frying oil were conducted with fairly high metal concentrations. However, current refining methods produce frying oils with very low starting concentrations of metals. Therefore, this study focused on the low levels of metals currently found in refined oils and included a higher concentration that might be reached when oils come in contact with foods or equipment.

6.2.4.1 Iron

Iron is a very common metal universally present in cooking utensils and equipment as well as in a variety of foods, so traces of iron are ubiquitously introduced into oils during frying. Indeed, levels of iron in all three oils (stripped, fresh and steady-state oil) are much higher than other metals (Table 12) and are in ranges expected to be catalytic. Thus, it is critical to evaluate effects of iron. Preliminary studies conducted in our lab adding 10 ppb iron to oils showed unexpected antioxidant rather than catalytic effects. Therefore, we increased concentrations to 100 ppb, 1 ppm and 10 ppm in the experiments reported here.

Table 10. Metal contents (ppb) of fresh, stripped and steady-state oils before frying (information provided by supplier, analyzed in a commercial laboratory by acid hydrolysis and inductively coupled plasma).

	ppb		
	Fresh	**Stripped**	**Steady-state**
Ti	1.5	1.8	1.6
V	1.5	1.9	1.3
Cr	11	60	23
Mn	0.9	2.4	1.1
Fe	12	64	33
Co	<5	<5	<5
Ni	2.2	3.1	3.3
Cu	4.5	16	7.5
Zn	5.3	2.8	3.3
Al	2.3	9.8	4.1

Ferrous chloride ($FeCl_2$) surprisingly decreased oxygen consumption in frying oils at all levels added, even when metal concentrations were increased to 10 ppm (Table 13). The oxygen consumption difference between iron treated samples and the control was statistically significant ($P<0.05$). This decrease may be attributed to conversion of radicals to ions by $FeCl_2$ at the levels evaluated in our study:

$$L\cdot + M^{n+} \rightarrow M^{(n+1)+} + L^{-}$$

The converted ions do not react with O_2, resulting in reduced O_2 consumption.

Table 11. Changes in O_2 consumption rate and total O_2 consumption in steady-state oil heated three hours with 100 ppb, 1 ppm and 10 ppm $FeCl_2$ added.

	Max rate (mmols O_2/min)	**O_2 consumption (mmols O_2/mol TAG)**
Control	0.018 ± 0.001	126.87 ± 2.01
100 ppb	0.012 ± 0.001	99.90 ± 0.66
1 ppm	0.013 ± 0.003	98.97 ± 13.80
10 ppm	0.011 ± 0.002	85.03 ± 13.80

Despite remarkable changes in oxygen consumption, addition of iron did not notably alter formation of the non-volatile products analyzed in this study (Figure 37). The most likely explanation is that iron catalyzes reactions or formation of products that are not being measured. One candidate reaction here might be oxygen insertion to generate ketones with no radicals, which is known to occur in aprotic media such as oils [95]. Increased ketones were observed in the trapped volatiles from these samples [79] but would not be detected in the non-volatile products assays. Such a reaction shift would also decrease oxygen consumption because a single oxygen atom is added rather than O_2. Clearly, the reaction chemistry is substantially more complex than either simple thermal scissions or accelerated radical chains of autoxidation. The metal actions are independent of thermal scissions, the products differ from thermal scission and autoxidation, and pathways of metal interactions in oils remain to be identified. Additional focused studies will be necessary to identify the multiple roles of metals in thermal degradation of oils.

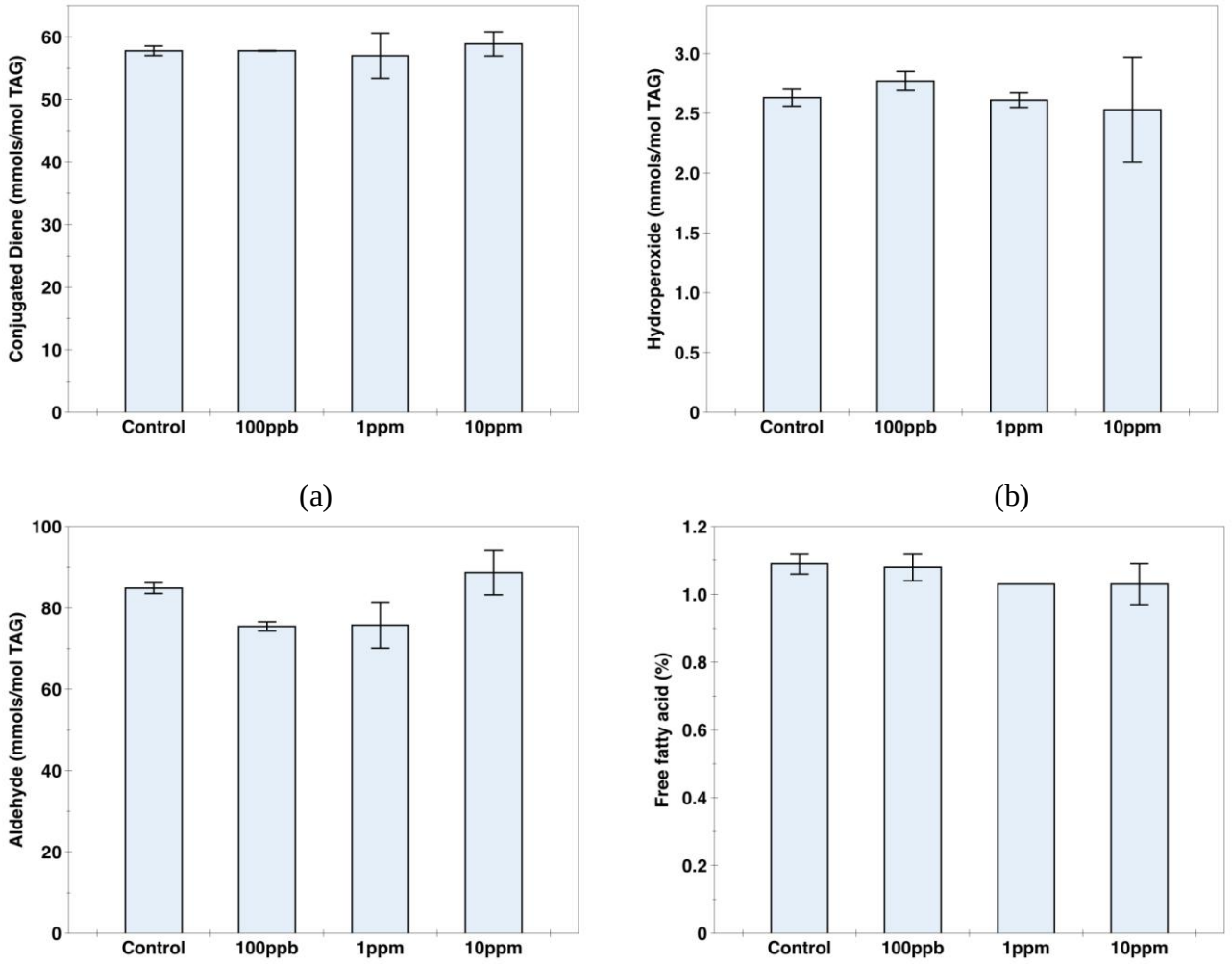


Figure 37. Levels of conjugated dienes (a), hydroperoxides (b), aldehydes (c), and free fatty acids (d) in steady-state oils heated for three hours with different levels of added iron.

6.2.4.2 Copper

Copper is another redox-active metal commonly present in frying oils. Like iron, addition of copper decreased rates and total amounts of oxygen consumption (Table 14). This again may be explained by Cu^{2+}-catalyzed conversion of scission radicals to ion, in this case by electron-transfer oxidation of alkyl radicals [96]:

$$L\cdot + M^{(n+1)+} \rightarrow M^{n+} + L^{+}$$

In contrast to iron, remarkable changes in some degradation products were observed with introduction of copper. Conjugated dienes remained unaltered, but hydroperoxides decreased, aldehydes increased, and free fatty acids decreased significantly in parallel with added copper concentrations (Figure 38). Cupric copper decomposes hydroperoxides to peroxyl radicals. With accumulation of locally high concentrations of $LOO^{\bullet}$, these radicals may recombine via the Russell mechanism to form alkoxyl radicals and then aldehydes. This proposed explanation is consistent with observed changes in hydroperoxide and aldehydes.

$$LOOH + Cu^{2+} \rightarrow LOO^{\bullet} + H^{+} + Cu^{+}$$

$$L_1OO^{\bullet} + {}^{\bullet}OOL_2 \rightarrow L_1\text{-}OOOO\text{-}L_2 \rightarrow O_2 + L_1O^{\bullet} + L_2O^{\bullet} \rightarrow L_1CHO + L_2CHO$$

Copper complexes with carboxylic acids [94] which might be the reason accounting for remarkable decrease in free fatty acids. However, removal of products already formed would imply that oxygen consumption had remained at the same or higher levels as controls, rather than the observed reduction of oxygen consumption. Thus, it is more likely that copper interferes with oxygenation of scission radicals to form terminal hydroperoxides and with oxidation of aldehydes to carboxylic acids.

Table 12. Changes in O_2 consumption rate and total O_2 consumption in steady-state oils heated three hours with 100 ppb, 1 ppm and 10 ppm $CuCl_2$ added.

$CuCl_2$	**Max rate (mmols O_2/min)**	**O_2 consumption (mmols O_2/mol TAG)**
Control	0.018 ± 0.001	128.24 ± 3.94
100 ppb	0.011 ± 0.000	92.46 ± 3.29
1 ppm	0.009 ± 0.001	72.95 ± 5.91
10 ppm	0.011 ± 0.001	77.59 ± 0.66

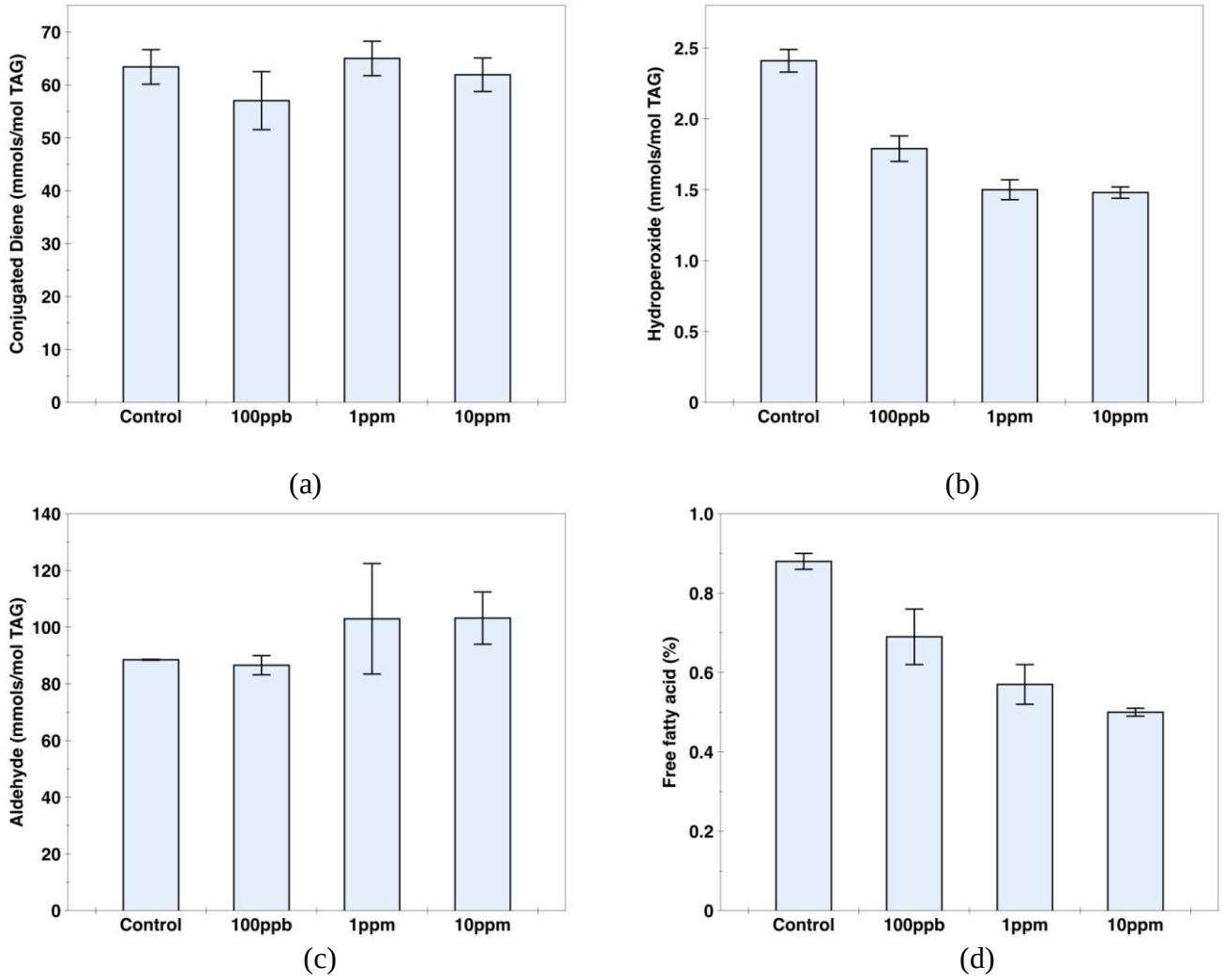


Figure 38. Levels of conjugated dienes (a), hydroperoxides (b), aldehydes (c), and free fatty acids (d) in steady-state oils heated for three hours with different levels of copper added.

6.2.4.3 Cobalt

Cobalt was also evaluated since it has been shown to catalyze oxidation of alkanes [97, 98], lipids [88, 99-101], and aldehydes [102, 103] and to complex with fatty acids [99]. Like iron and copper, the addition of cobalt remarkably altered oxygen consumption, both slowing the rate and reducing the amount of oxygen in the headspace (Table 15). The mechanism of this reduction may be metal complexation of radicals by cobalt [104]. The formed complex is stable and does not react with O_2.

$$L^{\bullet} + MA_n \rightarrow L\text{-}MA$$

Alterations in oxidation products were quite different from the other two metals. Cobalt added at all three levels significantly ($P<0.05$) reduced conjugated diene formation (Figure 39a), with no difference seen between concentrations. This may be caused by the same reason that reduced O_2 consumption, i.e. reduction in levels of active peroxyl radicals from thermal scissions correspondingly reduced formation of conjugated dienes. However, changes in hydroperoxides and aldehydes (Figure 39b and 39c) were not statistically significant, while free fatty acids increased notably with Co concentrations (Figure 39d). Oxidation of aldehydes to carboxylic acids, known to be a facile reaction with catalysts such as cobalt [105], would increase levels of fatty acids, although this must occur by a parallel and independent pathway from that forming aldehydes since their levels did not decrease. This points out, once again, that there are many unidentified reaction pathways active in oil degradation.

Copper had higher reactivity in heated oil than iron and cobalt. This may be attributed to different shell structures. Iron and cobalt react by outer sphere electron transfer in which electrons are donated or accepted directly. In contrast, copper reacts by

inner sphere electron transfer which requires the metal to form a complex with the substrate via a covalent bridge [94]. The more prominent effect of copper indicates that inner sphere electron transfer via oxygen or hydroperoxide complexes is more effective than direct electron transfer in altering the products analyzed in this dry bulk oil system. More detailed analyses are needed to identify pathways catalyzed and products generated by iron and cobalt.

Table 13. O_2 consumption rate and total O_2 consumption in steady-state oil heated three hours with 100 ppb, 1 ppm and 10 ppm $CoCl_2$ added.

	Max rate (mmols O_2/min)	**O_2 consumption (mmols O_2/mol TAG)**
Control	0.020±0.003	134.28±1.97
100 ppb	0.014±0.000	103.15±3.94
1 ppm	0.012±0.002	92.46±5.91
10 ppm	0.013±0.004	84.56±7.89

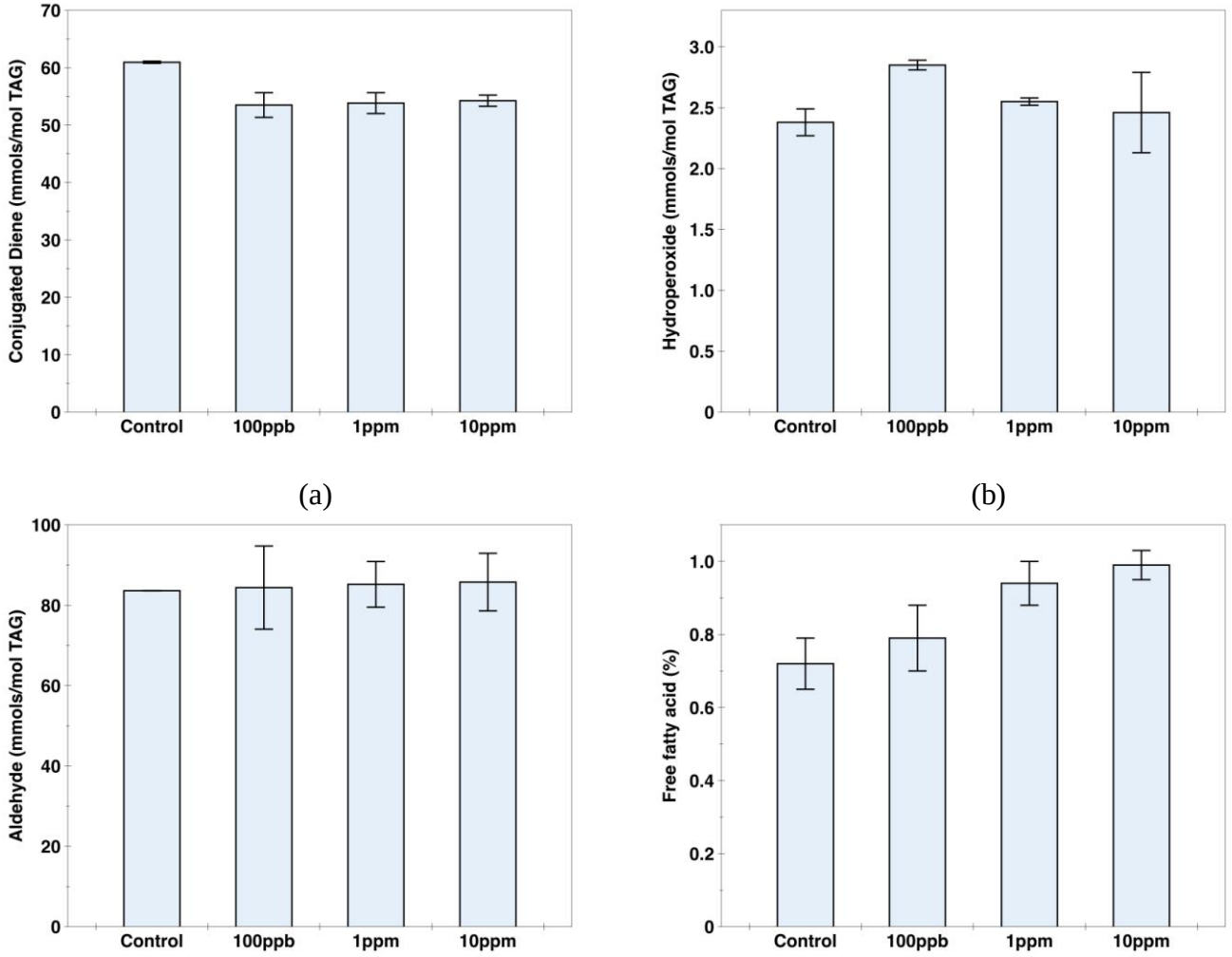


Figure 39. Levels of conjugated dienes (a), hydroperoxides (b), aldehydes (c), and free fatty acids (d) in oil added with different level of cobalt.

6.2.5 Effects of fatty acids

Free fatty acids are known to influence triacylglycerol oxidation, but no definitive data on mechanisms is available. In actual frying operations with food present, free fatty acids accumulate during heating when oil reaches Blumenthal's late "break-in" or "optimum" stages [2]. How these free fatty acids affect subsequent degradation of frying oils is important to identify. Both saturated fatty acids (stearic acid) and unsaturated fatty acids (oleic acid) were used in this study.

6.2.5.1 Saturated fatty acids (Stearic acid)

Addition of stearic acid to steady-state oil reduced oxygen consumption significantly (Table 16) but had little effect on products. Conjugated dienes slightly decreased and hydroperoxides slightly increased, while free fatty acids increased slightly but by substantially less than the amount added (0.11% and 0.15% increase for 0.25% and 0.5% stearic acid, respectively) (Figure 40). This pattern can be explained by metal complexing and by hydrogen bonding of the acid group to hydroperoxides, both of which would stabilize the hydroperoxides and prevent generation of conjugated dienes and subsequent chain reactions. The small reduction in oxygen consumption is consistent with the small disruptions of secondary oxidation.

Table 14. O_2 consumption rates and total O_2 consumption in oils different levels of stearic acid added.

Sample	**Max rate (mmols O_2/min)**	**O_2 consumption (mmols O_2/mol TAG)**
Control	0.019 ± 0.000	133.81 ± 0.00
0.05%	0.014 ± 0.001	115.69 ± 7.23
0.25%	0.014 ± 0.003	119.41 ± 15.11
0.50%	0.011 ± 0.003	121.73 ± 2.63

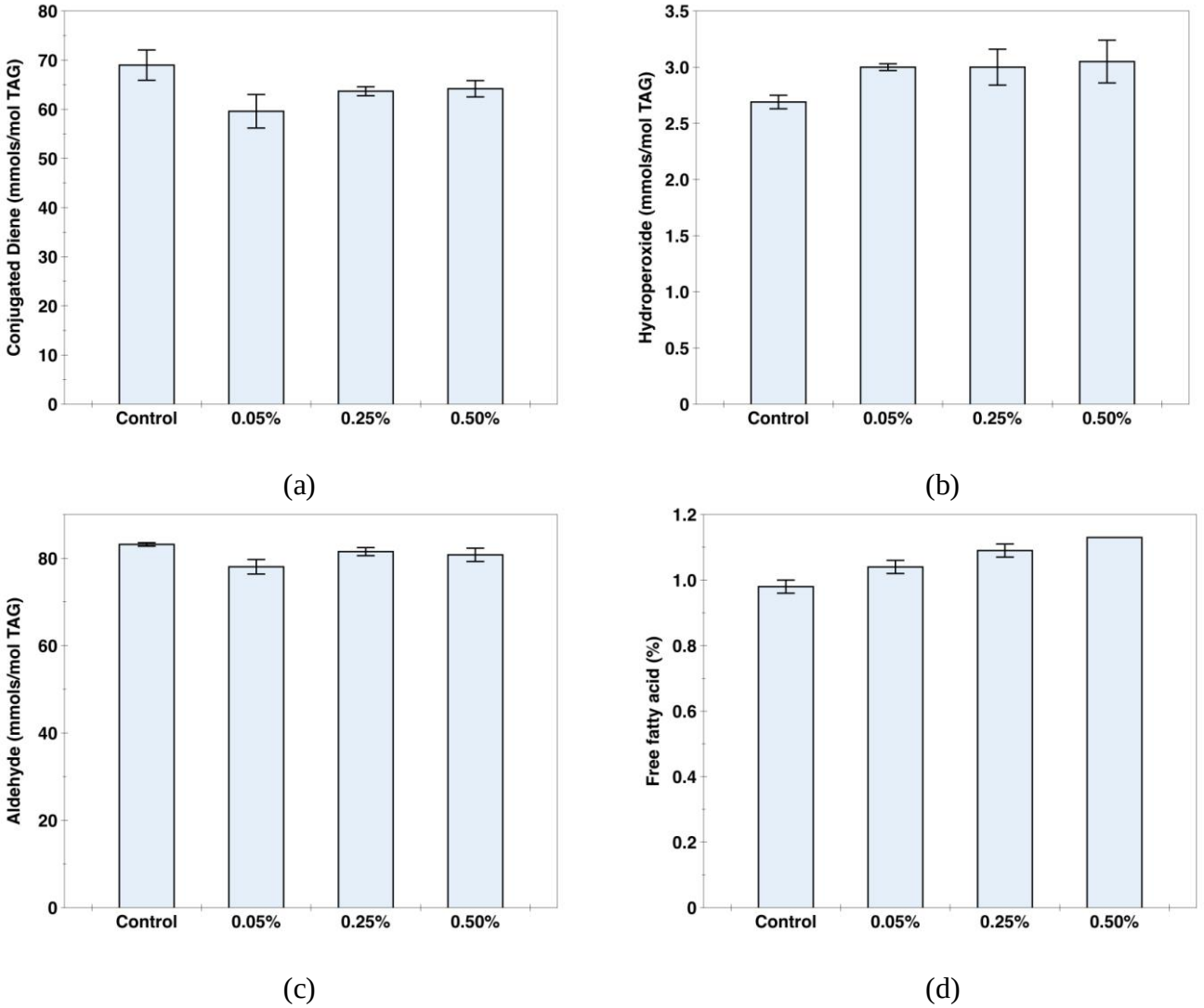


Figure 40. Levels of conjugated dienes (a), hydroperoxides (b), aldehydes (c), and free fatty acids (d) in steady-state oils heated three hours with different level of stearic acid added.

6.2.5.2 Effects of unsaturated fatty acids (Oleic acid)

Oleic acid was added to oil to determine contributions of double bonds in addition to the acid functional group. All three levels of oleic acid decreased oxygen consumption rate and amount significantly ($p<0.05$) (Table 17), although decreases were not much different from stearic acid if variation is considered. Effects on products were mixed (Figure 41). Conjugated dienes and aldehydes decreased, while hydroperoxides and fatty acids increased primarily at the highest level of oleic acid. The changes were quite similar to those observed in stearic acid, and none were remarkable.

Overall, effects of the double bond, if present, were subtle or may reside mainly in pathways not measured in this study. As with stearic acid, actions of the COOH group appear to be most important. Fatty acids modify oxidation by complexing metals in oil [99]; forming metal carboxylates increases solubility of metals in oils, may greatly enhance (or inhibit) their catalytic redox activity [106], and may mediate significant shifts in reaction pathways and products. We speculate that metal carboxylates converted radicals to ions, thus preventing their addition of oxygen and their abstraction of hydrogens to initiate radical chains. This would reduce oxygen consumption and conjugated dienes. COOH groups are known to hydrogen bond to hydroperoxides, stabilizing them against decomposition [107]. This action would account for increased hydroperoxide levels. Non-radical decomposition of hydroperoxides by acids, demonstrated in cellular systems [108], did not seem to occur in the heated oils. Metal carboxylates also catalyze oxidation of aldehydes to carboxylic acids [109], which would both decrease aldehydes and increase acids, as was observed..

Table 15. O_2 consumption rate and total O_2 consumption in steady-state oil heated with different levels of oleic acid added.

Oleic acid	**Max rate (mmols O_2/min)**	**O_2 consumption (mmols O_2/mol TAG)**
Control	0.019 ± 0.000	133.81 ± 0.00
0.05%	0.014 ± 0.001	109.65 ± 7.89
0.25%	0.014 ± 0.001	118.02 ± 1.31
0.50%	0.015 ± 0.001	113.37 ± 0.00

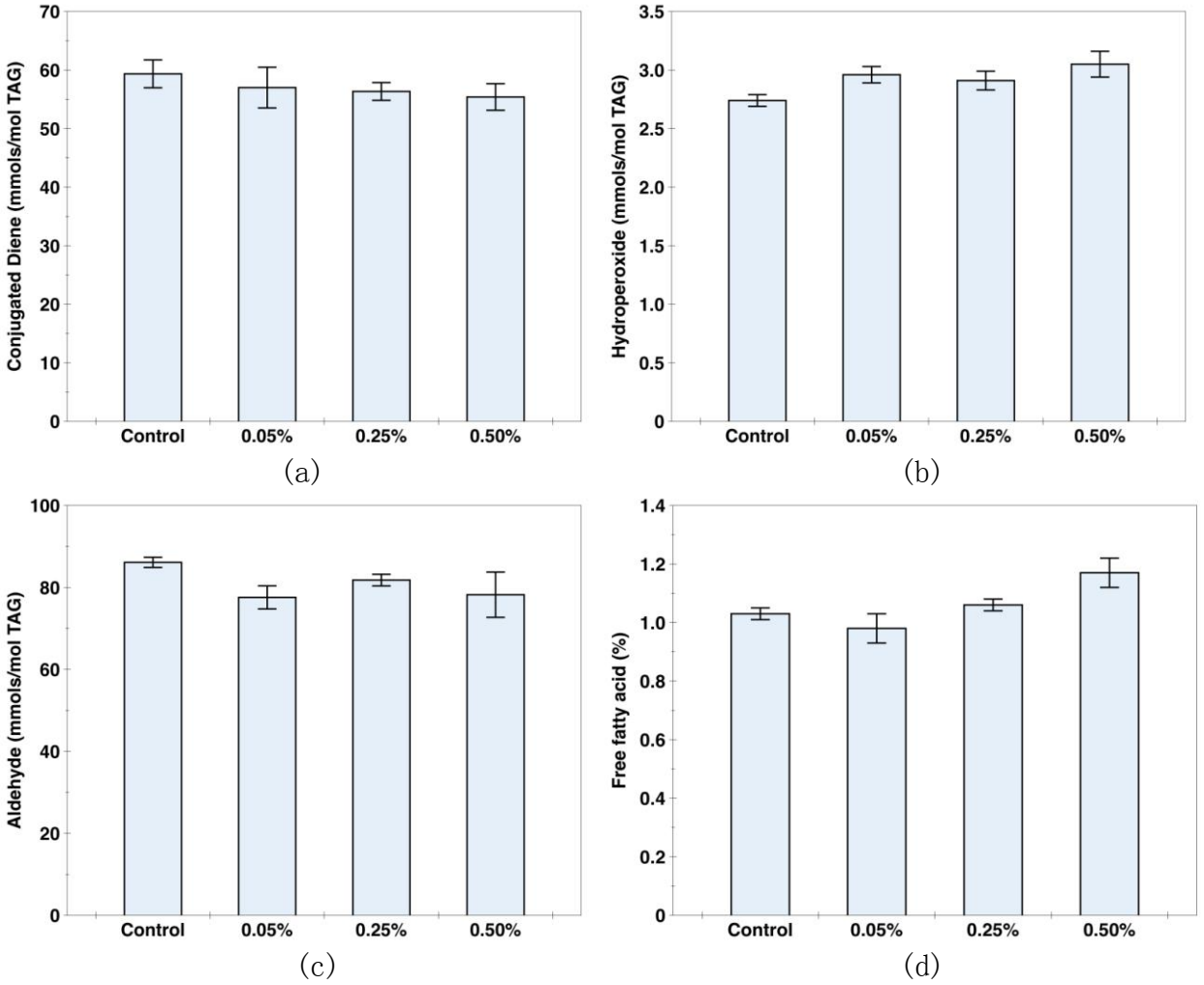


Figure 41. Levels of conjugated dienes (a), hydroperoxides (b), aldehydes (c), and free fatty acids (d) in steady-state oils with different levels of oleic acid added.

6.2.6 Effects of phospholipids

Traces of phospholipids remain after refining and may also be introduced into oil by foods. Phospholipids are known to exert both pro- and anti-oxidant effects through multiple mechanisms. For example, they may be preferentially oxidized and seed oxidation of triacyglycerols [110], yet this same characteristic might also impose an antioxidant effect with phospholipids sacrificing themselves to prevent the oxidation of TAGs. Phosphatidylcholine decomposes hydroperoxides to non-radical products, thereby blocking propagation of radical chains, and antioxidant action. Phospholipids also bind metals and water and move these molecules between phases, altering their reactivity.

Most of above-mentioned research was conducted at room temperature; studies on phospholipid effects under heated conditions are scarcely found. In the present study, addition of 0.02% and 0.05% of phosphatidylcholine distearoyl to oils heated at 180°C didn't affect maximum oxygen consumption rate, but slightly decreased apparent O_2 consumption (Table 18). Corresponding effects on oxidation products were also minimal. Although we expected reduction of hydroperoxides by non-radical decomposition through the nucleophilic N^+ in phosphatidylcholine (Figure 42), no change in hydroperoxide levels was observed (Figure 43b). Aldehydes were similarly unchanged. The only change in treated samples wwas observed in free fatty acids level. Addition of 0.05% di-St PC sample increased FFA about 0.1% (Figure 43d); yet, this increase may well be introduced by the diacylglycerol in phosphatidylcholine structure. Overall, results indicated that phospholipids at the levels tested in this study have little or no catalytic or protective effects on major oxidation in oils when present alone, at least in terms of the characteristics measured.

Table 16. O_2 consumption rates and total O_2 consumption in oil heated for three hours with distearoyl phosphatidylcholine.

Sample	Max rate (mmols O_2/min)	O_2 consumption (mmols O_2/mol TAG)
Control	0.019 ± 0.000	134.28 ± 0.66
0.02%	0.019 ± 0.000	124.99 ± 3.29
0.05%	0.018 ± 0.002	124.99 ± 8.54

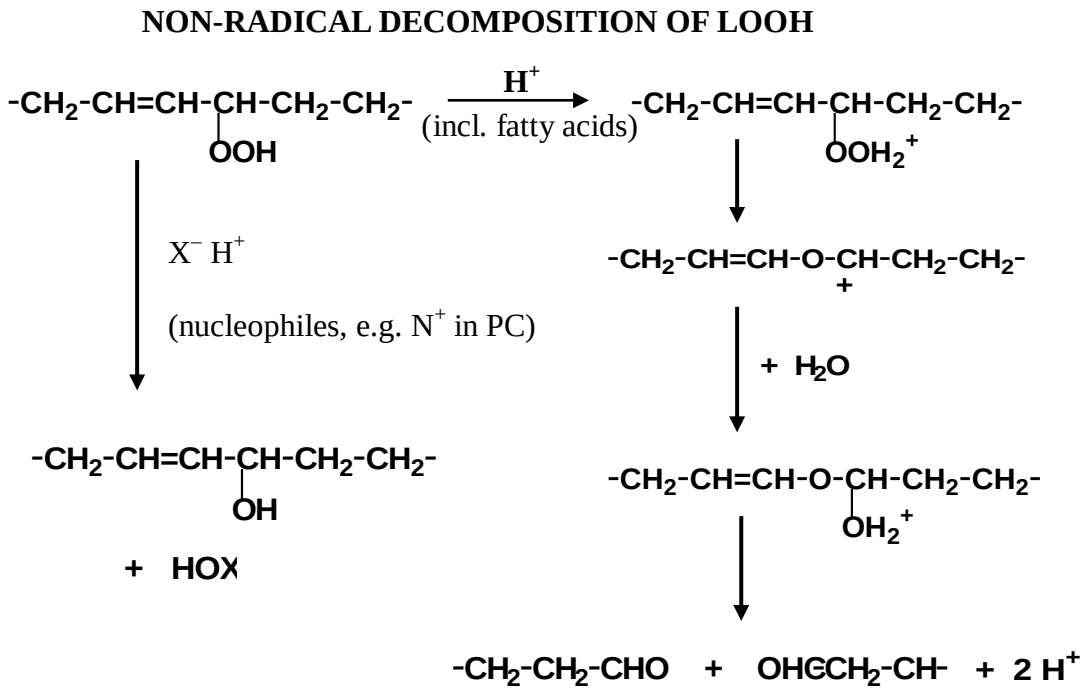


Figure 42. Non-radical decomposition of hydroperoxides by nucleophiles such as phosphatidylcholine and acids. Redrawn from [108].

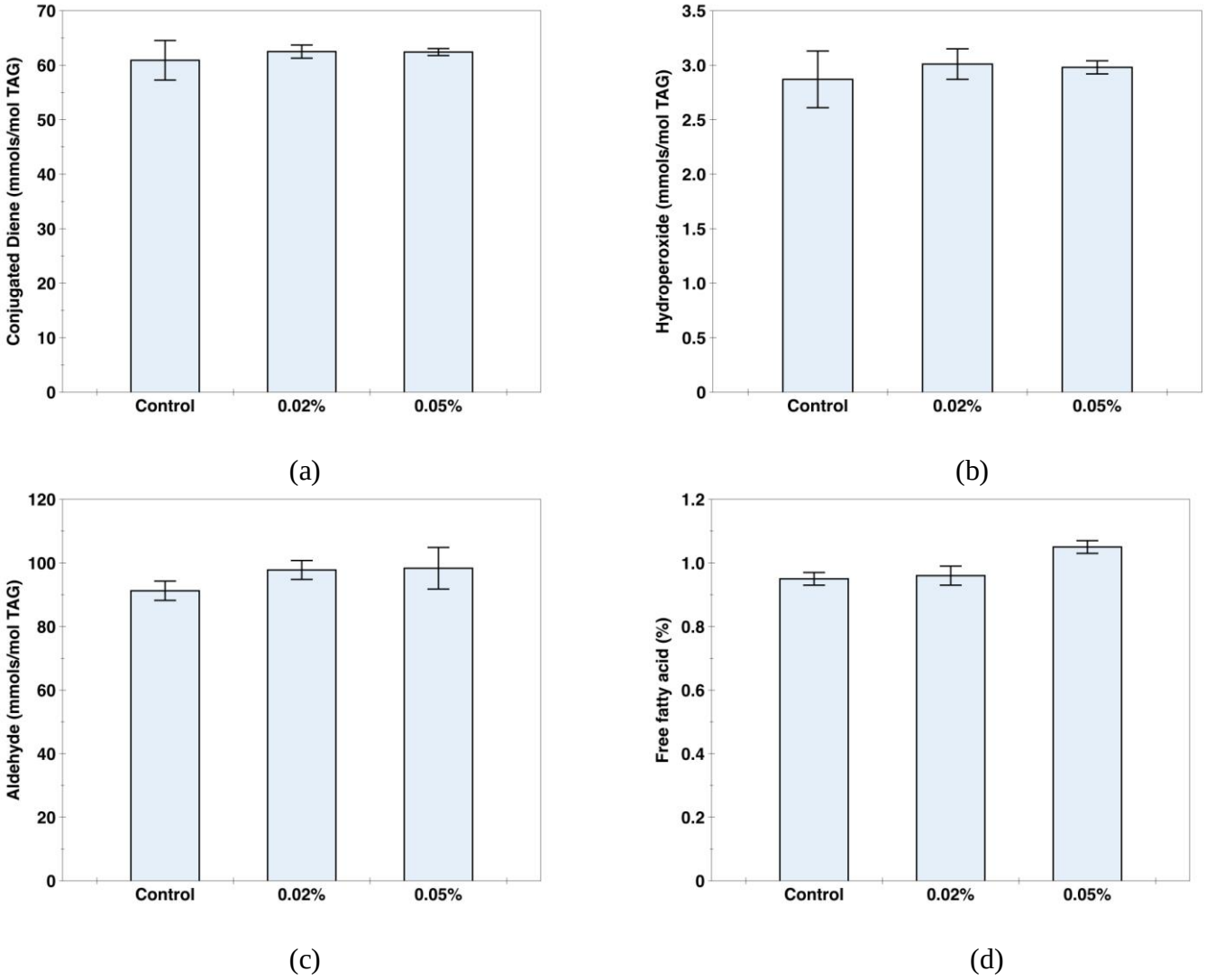


Figure 43. Levels of conjugated dienes (a), hydroperoxides (b), aldehydes (c), and free fatty acids (d) in steady-state oil heated for three hours with two levels of distearoyl phosphatidylcholine.

6.3 EFFECTS OF FACTOR COMBINATIONS

6.3.1 Effects of metals + water

Metals and water added together significantly ($p<0.05$) reduced oxygen consumption compared to neat control (Table 19). Compared to $FeCl_2$ added samples, however, oxygen consumption values were very close, suggesting minimal influence from water. Of the degradation products measured, conjugated dienes, hydroperoxides and aldehydes didn't change very much; the only significant difference was observed in fatty acids (Figure 44). Overall, water didn't affect the behavior of $FeCl_2$ to any notable extent.

Table 17. Oxygen consumption rates and total O_2 consumption of oil heated for three hours with 2% water and variable amounts of $FeCl_2$.

	Max rate (mmols O_2/min)	**O_2 consumption (mmols O_2/mol TAG)**
Neat Control	0.018 ± 0.001	127.87 ± 3.94
H_2O Control	0.012 ± 0.001	73.88 ± 1.17
100 ppb Fe	0.012 ± 0.000	93.66 ± 3.62
1 ppm Fe	0.012 ± 0.001	89.17 ± 10.65
10 ppm Fe	0.011 ± 0.002	81.09 ± 3.34

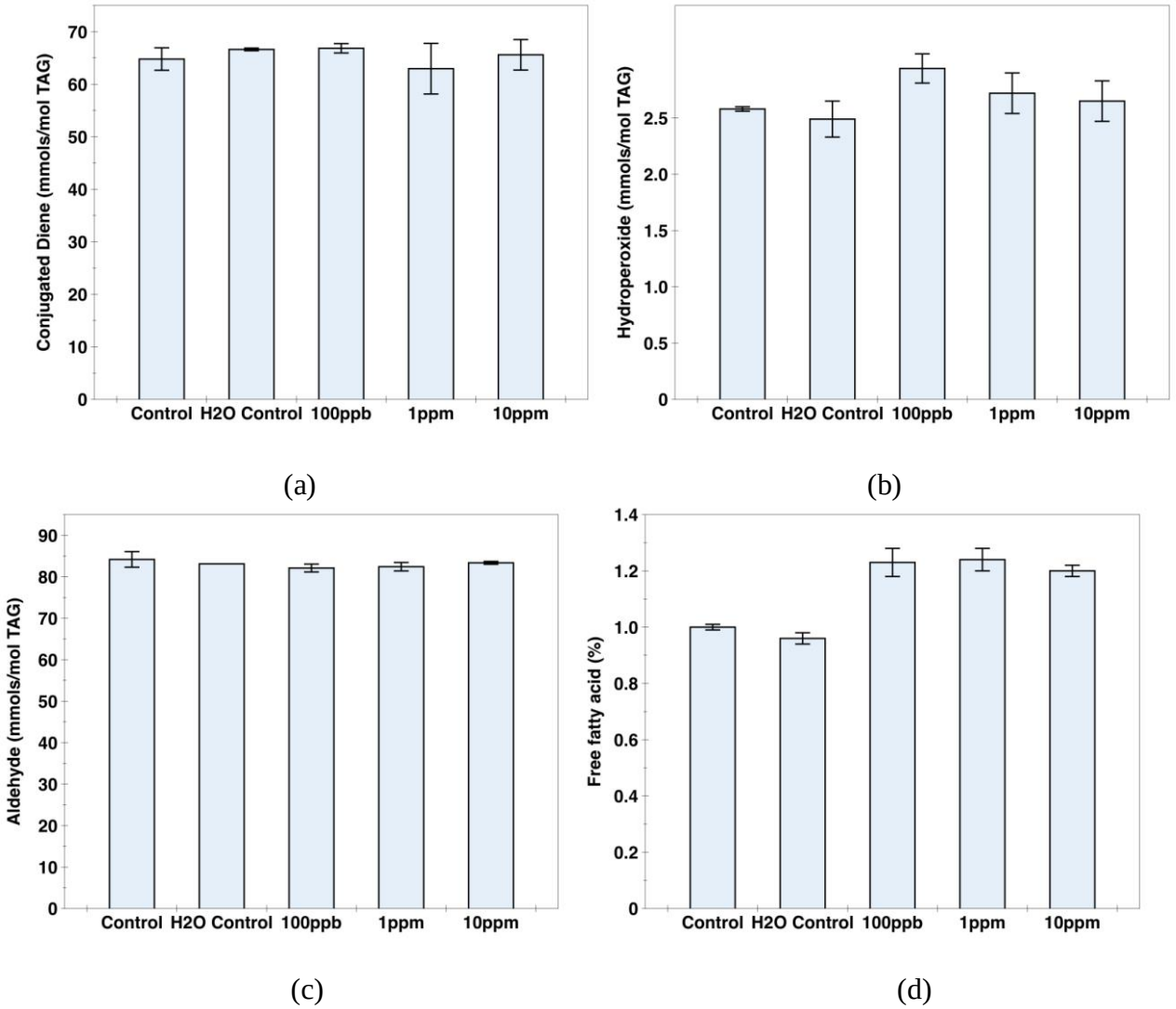


Figure 44. Levels of conjugated dienes (a), hydroperoxides (b), aldehydes (c), and free fatty acids (d) in steady-state oils heated three hours with 2% water and variable amounts of $FeCl_2$.

6.3.2 Effects of fatty acids + water

Oleic acid reduced oxygen consumption; water alone reduced oxygen consumption even more. Net oxygen consumption rates for the combination of factors were slightly less than the average of the two (Table 20), suggesting that water played a somewhat more dominant role than fatty acids. In contrast, the oxidation products measured were barely altered by the combination of factors (Figure 44). Conjugated dienes and aldehydes remained nearly constant under all conditions (Figure 44a and c), while hydroperoxides decreased marginally (Figure 44b). Free fatty acid levels initially dropped with low levels of added oleic acid, but then increased linearly with additional oleic acid (Figure 44d), and the increases were larger than that observed in single factor studies. When water is added by itself, it evaporates rapidly into the headspace and does not remain in the oil long enough to induce hydrolysis, hence little change in fatty acids levels occurs. Fatty acids present at the same time may bind water, retaining it in the oil. This bound water may then catalyze hydrolysis, which in turn increases fatty acid levels.

COOH metal binding and stabilization of LOOH by hydrogen bonding are very likely active in this system, as with oleic acid alone. Opposite and counterbalancing effects of these two actions may contribute to the apparent lack change in oxidation products. However, the mismatch in oxygen consumption and oxidation products suggests another possibility, that the dominant pathway(s) result in products other than those analyzed, e.g. ketones and epoxides, or dimers and polymers. These alternate degradation pathways need to be analyzed and documented before potentially erroneous assumptions are made regarding interactive effects of water and fatty acids.

Table 18. Oxygen consumption rate and total O_2 consumption of steady-state oil heated three hours with variable amounts of oleic acid and 2% water.

	Max rate (mmols O_2/min)	**O_2 consumption (mmols O_2/mol TAG)**
Control	0.018 ± 0.002	126.84 ± 9.86
H_2O Control	0.011 ± 0.001	72.95 ± 13.80
+ 0.05% oleic acid	0.013 ± 0.001	82.71 ± 2.63
+ 0.25%	0.012 ± 0.001	91.54 ± 9.53
+ 0.5%	0.012 ± 0.001	90.14 ± 3.94
Oleic Acid alone		
0.05%	0.014 ± 0.001	109.65 ± 7.89
0.25%	0.014 ± 0.001	118.02 ± 1.31
0.50%	0.015 ± 0.001	113.37 ± 0.00

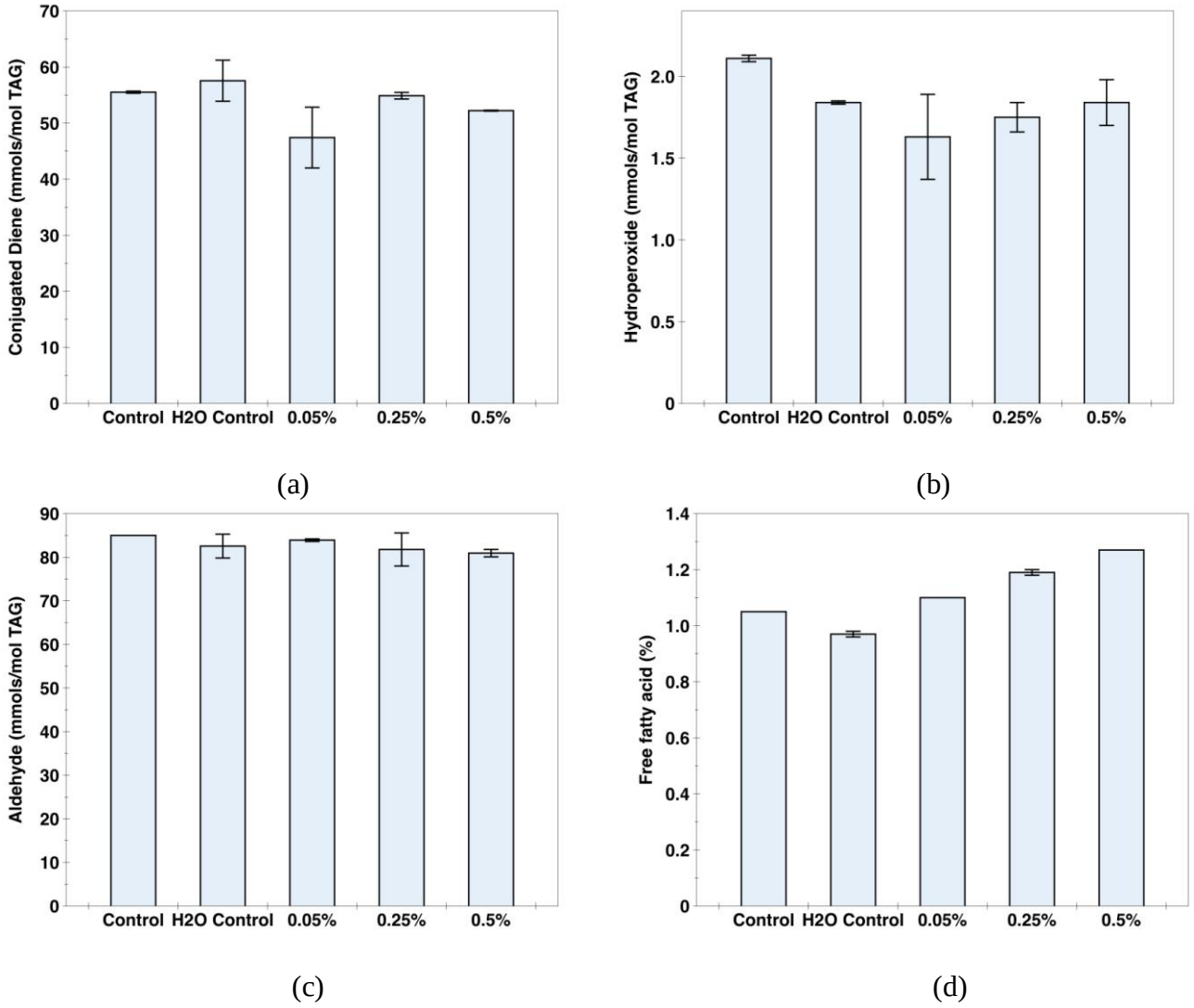


Figure 45. Levels of conjugated dienes (a), hydroperoxides (b), aldehydes (c), and free fatty acids (d) in oil heated three hours with 2% water and variable amounts of oleic acid.

6.3.3 Effects of fatty acids + metals

Since it is known that fatty acids complex metals and these complexes can both enhance and inhibit oxidation, we added both factors to oils being heated to investigate whether these interactions occur in frying oils and which effect would dominate.

When both iron ($FeCl_2$) and oleic acid were present, rates and amounts of oxygen consumption were significantly reduced ($p<0.05$) over oleic acid alone (Table 20) but were nearly the same as iron alone. Also, reduction in oxygen consumption increased proportionally with iron concentration, indicating that there was no synergistic effect between oleic acid and iron on reactions responsible for oxygen consumption and that iron played the dominant role in controlling oxygen consumption.

The contrary situation was observed in the products where oleic acid played the major role (Figure 45). When added alone, oleic acid only mildly decreased conjugated dienes and aldehydes, and increased free fatty acids (Chapter 6). In the 2-way combination of metal+oleic acid, the same trend was observed yet the difference became more significant ($p<0.05$), and the changes were not concentration dependent. Therefore, it can be concluded at this point that co-existence of iron and oleic didn't change their respective behavior but one dominates the other in different aspects：iron controlled oxygen consumption process and oleic acid played a manipulative role in product formation. As to why they control different things we don't know yet, but this is again suggesting that oxygen consumption is a different process from the products that we measured. The extent of alterations in the products was very close to single factors, suggesting the complex formed between metal and fatty acids, if there's any, is not exerting different behavior from individual components.

Table 19. Oxygen consumption rate and total O_2 consumption in steady-state oil heated three hours with 0.5% oleic acid and variable amounts of $FeCl_2$.

	Max rate (mmols O_2/min)	**O_2 consumption (mmols O_2/mol TAG)**
Blank Control	0.017 ± 0.001	125.45 ± 0.00
0.50% Oleic acid	0.015 ± 0.001	113.37 ± 0.00
Fe alone		
100 ppb	0.012 ± 0.001	99.90 ± 0.66
1 ppm	0.013 ± 0.003	98.97 ± 13.80
10 ppm	0.011 ± 0.002	85.03 ± 13.80
0.50% Oleic acid + Fe		
100 ppb	0.014 ± 0.000	103.71 ± 1.71
1 ppm	0.012 ± 0.000	100.82 ± 3.61
10 ppm	0.011 ± 0.001	85.03 ± 3.61
Fe + water		
100 ppb Fe	0.012 ± 0.000	93.66 ± 3.62
1 ppm Fe	0.012 ± 0.001	89.17 ± 10.65
10 ppm Fe	0.011 ± 0.002	81.09 ± 3.34

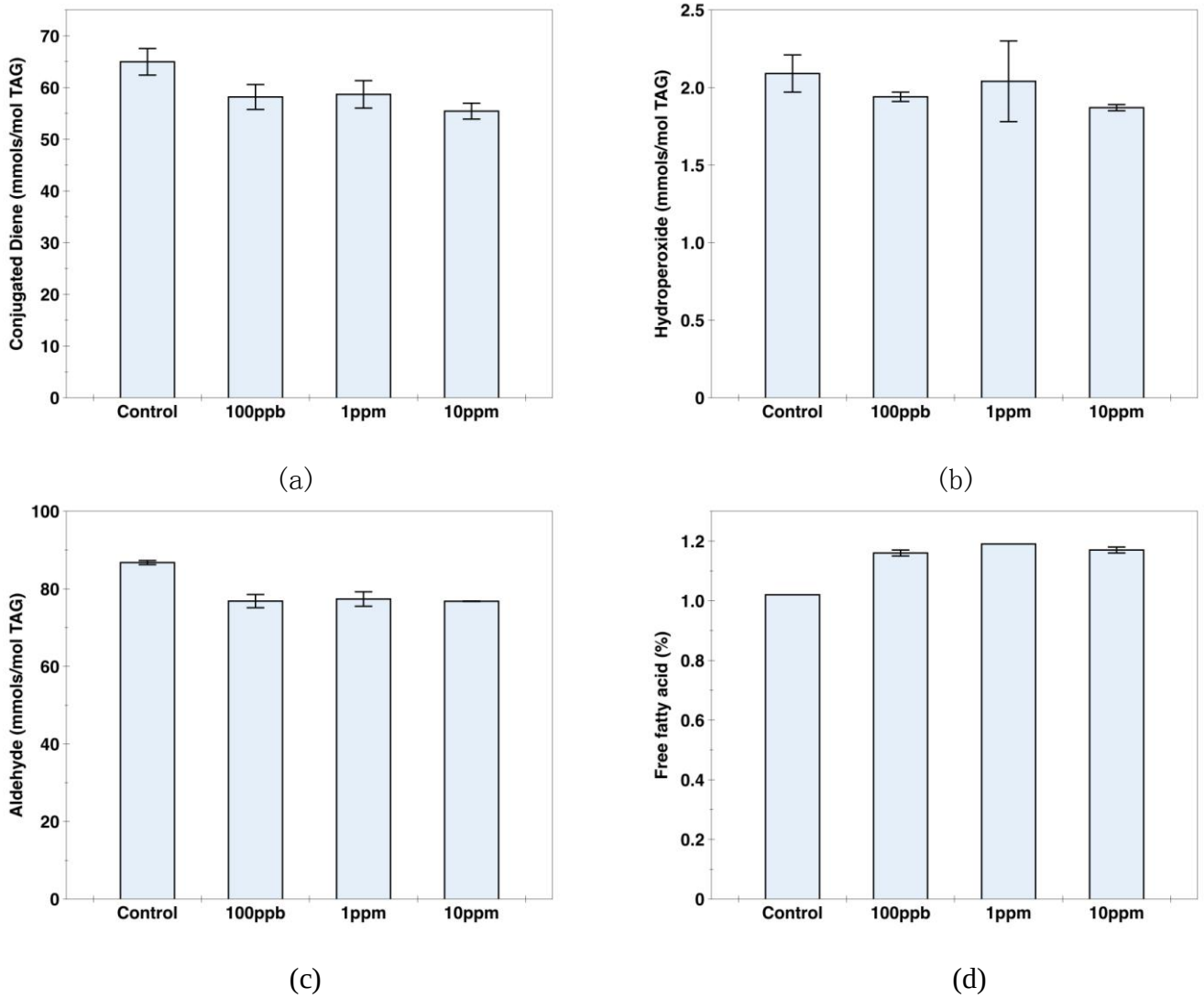


Figure 46. Levels of conjugated dienes (a), hydroperoxides (b), aldehydes (c), and free fatty acids (d) in oil heated three hours with 0.5% oleic acid and variable amounts of $FeCl_2$.

6.3.4 Effects of fatty acids + metals + water

Combination of 0.5% oleic acid, 2% water and different concentrations of iron were tested in this interaction. Adding oleic acid to water and iron had no effect on rates of initial oxygen consumption, which remained the same as water and iron alone, and decreased very slightly with increased iron levels, the same as iron alone. However, total oxygen consumption levels were neither additive nor averages, indicating complex interactions among the three factors. Comparing Fe vs Fe-Oleic acid, total oxygen consumption was less than the average of the two factors alone, and the difference increased with the Fe concentration. This pattern supports a dominance of Fe effects, although the nature of the effects or interactions cannot yet be identified. Comparing Fe and Fe+ water, total oxygen consumption was greater than the average of the two factors alone, again supporting a dominant role for Fe. However, for the three-way combination, total oxygen consumption (OC) was less than the average for the two lower iron concentrations but equal to the average for 10 ppm iron, and these OC levels were lower than the Fe+water levels for the lowest Fe concentration, but higher than the Fe+water level at the highest Fe concentration. Furthermore, the difference in OC with Fe concentration was eliminated in the three-way interaction. Altogether, these results all suggest that oleic acid was indeed interacting with Fe and water, and the interaction increased with Fe concentration.

Interesting changes were observed in the products (Figure 46). Conjugated dienes increased, unlike any of the single or two-way factors except water and oleic acid alone, and in the three way interaction, the increase was greater. Hydroperoxides decreased to about the same extent as other two-way interactions, but in distinct contrast to increases

with iron and oleic acid alone. Aldehydes decreased more than in any single factor or two-way interaction, and the decreases were dependent on metal concentration. For example, two-way interaction of 0.5% oleic + water decreased aldehyde levels by about 4.8% (percentage change based on control), but in the three-way interaction, aldehyde concentrations dropped by 12.2%, 21.7%, and 29.4% with addition of 100 ppb, 1 ppm and 10 ppm $FeCl_2$, respectively. In parallel, fatty acid concentrations increased much more than in other systems. 0.5% Oleic acid + water increased free fatty acid concentrations by 20.9% compared to control, but addition of iron increased fatty acid levels still further, by 28.7%, 34.0% and 41.5% (for 100 ppb, 1 ppm, 10 ppm, respectively). Therefore, the involvement of water totally changed the way iron and fatty acids interact with each other and the system. The product patterns suggest that the three-way combination of Fe-oleic acid-water activated the iron to initiate radical chains more effectively and catalyze oxidation of aldehydes to carboxylic acids more rapidly. One way that this may occur is that amphiphilic fatty acids act as emulsifiers in the presence of water and facilitate interaction between water-based iron and the oil phase, which explains the concentration dependency of products on metal. Alternatively, the three-way interaction may create a redox-active complex in which iron is stabilized in its ferric form, making it a stronger oxidizing agent. Additional research is needed to identify the detailed mechanisms of interactions between water, iron, oleic acid, and oil in this mixed system.

Table 20. Oxygen consumption rates and total O_2 consumption in steady-state oil heated three hours with 0.5% oleic acid, 2% water, and variable levels of $FeCl_2$.

	Max rate (mmols O_2/min)	**O_2 consumption (mmols O_2/mol TAG)**
Control	0.018 ± 0.000	131.03 ± 2.63
H_2O Control	0.012 ± 0.001	82.24 ± 0.66
0.50% oleic acid	0.015 ± 0.001	113.37 ± 0.00
$FeCl_2$ alone		
100 ppb	0.012 ± 0.001	99.90 ± 0.66
1 ppm	0.013 ± 0.003	98.97 ± 13.80
10 ppm	0.011 ± 0.002	85.03 ± 13.80
Oleic acid +Fe		
100 ppb	0.014 ± 0.000	103.71 ± 1.71
1 ppm	0.012 ± 0.000	100.82 ± 3.61
10 ppm	0.011 ± 0.001	85.03 ± 3.61
Three-way combination		
100 ppb	0.013 ± 0.000	90.61 ± 0.99
1 ppm	0.012 ± 0.000	90.61 ± 3.61
10 ppm	0.011 ± 0.001	90.14 ± 0.66

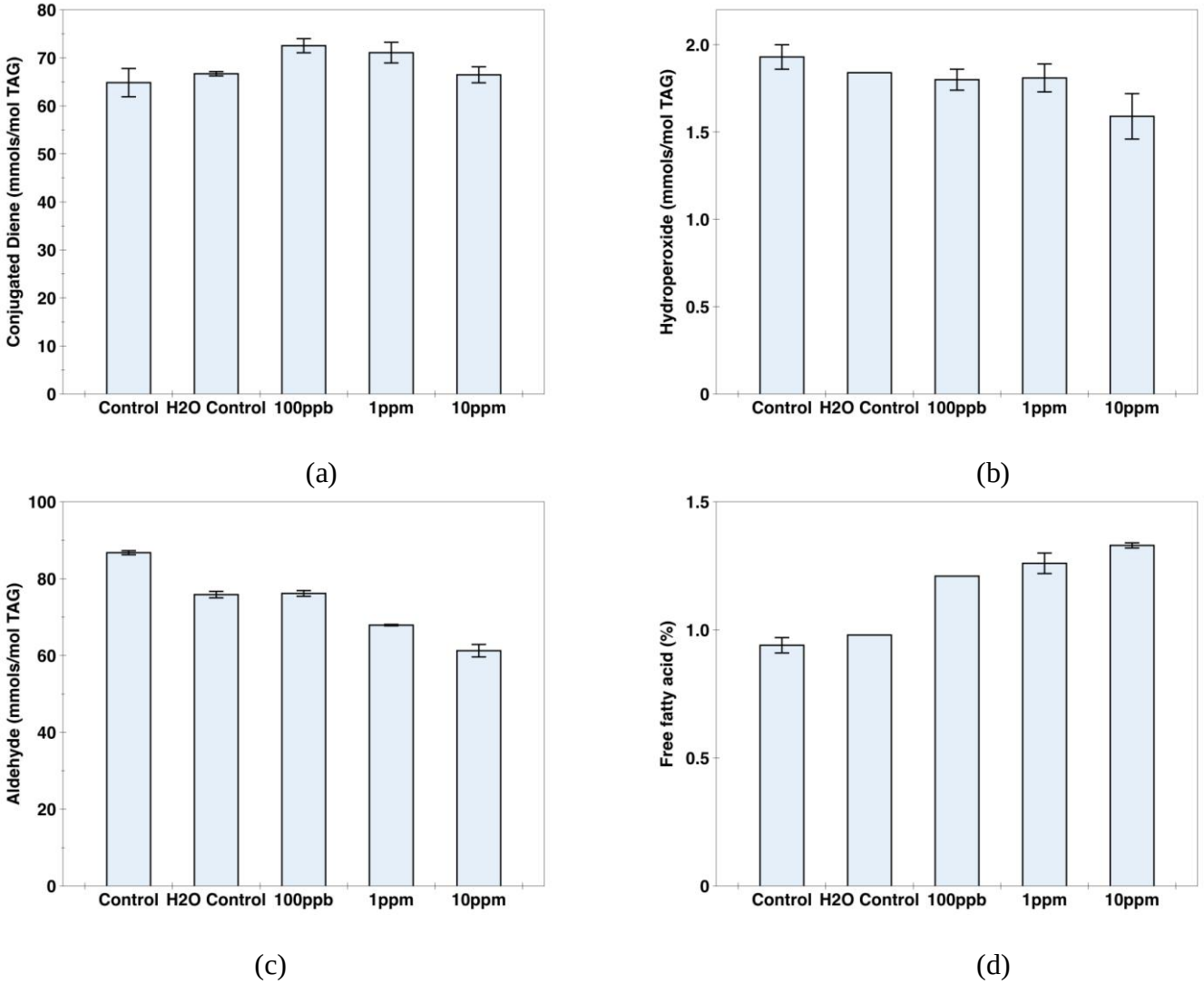


Figure 47. Levels of conjugated dienes (a), hydroperoxides (b), aldehydes (c), and free fatty acids (d) in oils heated for three hours with 0.5% oleic acid, 2% water, and variable levels of $FeCl_2$.

6.3.5 Effect of phospholipids + water

Water by itself reduced O_2 consumption greatly. With phospholipid present at the same time, this antioxidant of water was inhibited, supported by an increase in O_2 consumption compared to water control (Table 22). Data here does not match data in Master Table, which says PC+water increases both rate and total oxygen consumption. Which is correct? Phospholipid has strong ability to bind water; thus, there would be less water being flashed off into the headspace to interfere with O_2 consumption.

Water alone reduced oxygen consumption; phosphatidylcholine alone had little effect on oxygen consumption. In combination, don't know what to say here – water alone and PC water controls show opposite effects but should be same. Please check data.

Distearoyl phosphatidylcholine (diSt-PC) + water decreased conjugated diene concentration in the oil (Figure 47a). The oil blends used in this study contained 800 ppm tocopherol. Koga et al. [111] found phospholipids enhanced radical scavenging activity of Vitamin E, thus inhibiting chain initiation, when water-soluble radical initiators were used, but not with lipid-soluble initiators. This may explain why PC didn't affect conjugated dienes when added alone but inhibited their formation when water was involved. Alternatively, the PC-water combination may have enhanced conversion of initial products (thus reducing conjugated dienes) or may have catalyzed alternate reactions because hydroperoxides, aldehydes, and free fatty acids all increased (Figure 47), the latter two significantly ($p<0.05$). These changes were not observed when phospholipid was added by itself, suggesting addition of water change the behavior of phospholipids, making it to be pro-oxidant. Master table says PC alone showed increases in all measures, including CD, although difference was less than in combination. Need to

reconcile data before revising this section. Master table indicates PC was most active catalyst alone and even more so with water.

Table 21. Oxygen consumption rate and total O_2 consumption of steady-state oil heated for three hours with 2% water and variable levels of distearoyl phosphatidylcholine added.

	Max rate (mmols O_2/min)	**O_2 consumption (mmols O_2/mol TAG)**
Control	0.017±0.002	126.38±11.83
H_2O Control	0.012±0.002	72.49±13.14
0.02%	0.016±0.000	100.83±4.93
0.05%	0.014±0.000	106.40±2.30

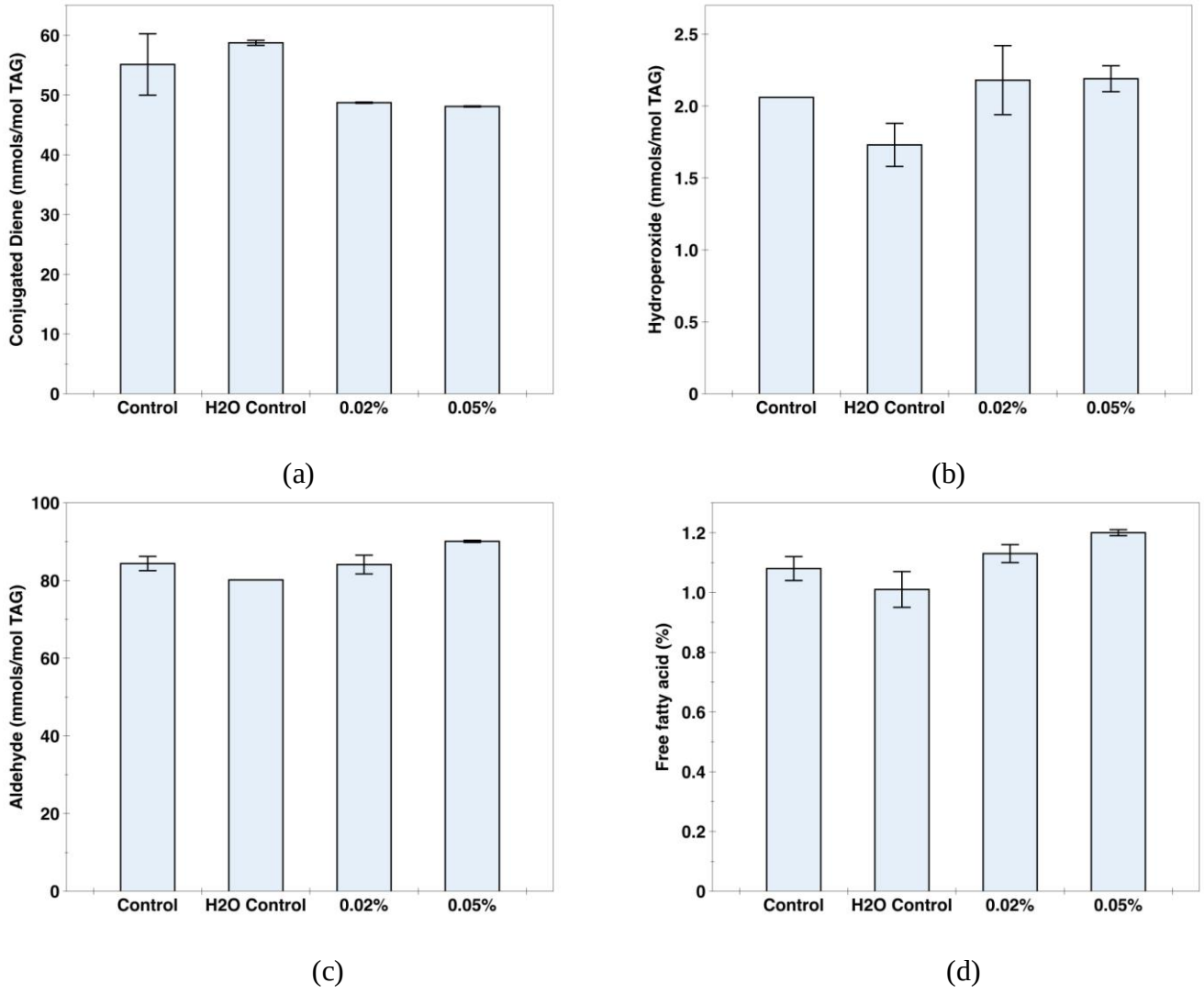


Figure 48. Levels of conjugated dienes (a), hydroperoxides (b), aldehydes (c), and free fatty acids (d) in steady-state oils heated for three hours with 2% water and variable levels of phospholipid.

6.3.6 Effect of phospholipids + metals

O_2 consumption rate and amount stayed between that of $FeCl_2$ and PC-di-St alone (Table 23). The fact that the addition of phospholipid increased oxygen consumption relative to iron alone indicates that the collective presence of the two factors weakened antioxidant effects of iron on O_2 consumption.

Evidence for interaction of phospholipid and iron was stronger in the change in patterns of lipid oxidation products (Figure 49). For iron alone, there was little change in CD or PV, decrease then increase in aldehydes, and slight decrease in fatty acids. PC alone had no notable effect on any product. In contrast, when phospholipid and iron were combined, CD decreased then increased with iron concentration; peroxides decreased mildly with iron, and aldehydes and fatty acids increased. CD and PV levels were lower than for either factor alone. Thus, interaction between iron and PC induced a marginal decrease in early oxidation product levels and shifted degradation pathways leading to secondary products. The fatty acid increases may results from iron binding to PC in preference to fatty acids, leaving the latter free and detectable.

Table 22. Oxygen consumption rate and total O_2 consumption of steady-state oil heated for three hours with 0.05% distearoyl phosphatidylcholine and variable levels of iron.

Sample	**Max rate (mmols O_2/min)**	**O_2 consumption (mmols O_2/mol TAG)**
Control	0.017 ± 0.003	127.77 ± 9.86
DiSt-PC 0.05%	0.018 ± 0.002	124.99 ± 8.54
$FeCl_2$		
100 ppb	0.012 ± 0.001	99.90 ± 0.66
1 ppm	0.013 ± 0.003	98.97 ± 13.80
10 ppm	0.011 ± 0.002	85.03 ± 13.80
DiSt-PC + $FeCl_2$		
100 ppb	0.016 ± 0.000	111.98 ± 2.96
1 ppm	0.016 ± 0.000	114.76 ± 2.30
10 ppm	0.016 ± 0.000	114.30 ± 2.63

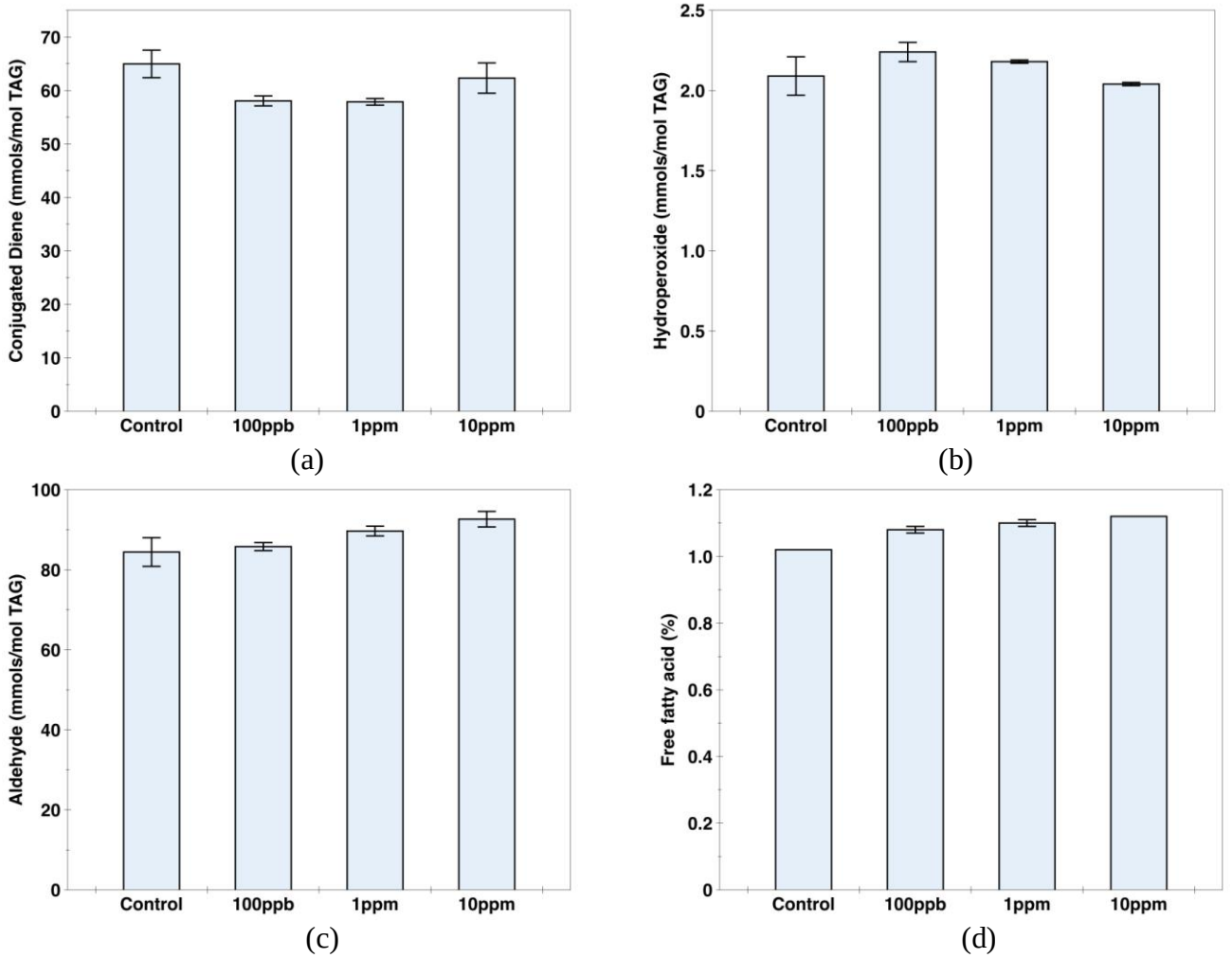


Figure 49. Levels of conjugated dienes (a), hydroperoxides (b), aldehydes (c), and free fatty acids (d) in steady-state oil heated for three hours with 0.05% distearoyl phosphatidylcholine and variable levels of iron.

6.3.7 Effects of phospholipids + metals + water

Phosphate group of phospholipids binds water. Therefore, the combination of metal, water and phosphatidylcholine is expected to have a great influence on oil stability. Yet, whether this hydration water is protective or detrimental is not known [110].

Oxygen consumption reduced with the introduction of water compared to 2-way combination under dry conditions (Table 24), indicating a dominant role of water on O_2 consumption. So far, of all combination tests, water always played the most important role in altering the amount and rate of O_2 being consumed. In terms of degradation products, decreases in hydroperoxide and aldehyde and an increase in free fatty acid were detected (Figure 50). Changes in hydroperoxides and fatty acids were much more significant than in the dry system. Another interesting observation would be that when comparing with products changes in Oleic+Fe+water combination, very similar patterns were observed. This is strong evidence of interaction between multiple factors facilitated by amphiphilic fatty acids and phospholipids. Especially, concentration dependence of degradation products on metal, as well as an absence of notable effects on products by 2-way combination of metal+water suggested a promoted interaction between water and metal through participation of fatty acids and phospholipids.

Current evidence suggested that phospholipids can be both pro- and antioxidant depending on the system [112]; yet the underlying causes are not clearly understood. Our study indicated that when working alone or with metal in a dry oil system, PC is not affecting the oil to a significant extent; however when water is introduced, hydration effect of phospholipids facilitated interaction of substances present in the oil.

Table 23. Oxygen consumption rate and total O_2 consumption of steady-state oil heated for three hours with 2% water, 0.05% phospholipid, and variable levels of $FeCl_2$.

Sample	Max rate (mmols O_2/min)	O_2 consumption (mmols O_2/mol TAG)
Control	0.018 ± 0.001	131.95 ± 1.31
H_2O control	0.012 ± 0.001	82.24 ± 0.66
PCdi-St + $FeCl_2$		
100ppb	0.016 ± 0.000	111.98 ± 2.96
1ppm	0.016 ± 0.000	114.76 ± 2.30
10ppm	0.016 ± 0.000	114.30 ± 2.63
PC + Fe + H_2O		
100ppb	0.015 ± 0.000	97.11 ± 6.90
1ppm	0.012 ± 0.000	93.86 ± 5.91
10ppm	0.012 ± 0.000	95.72 ± 1.97

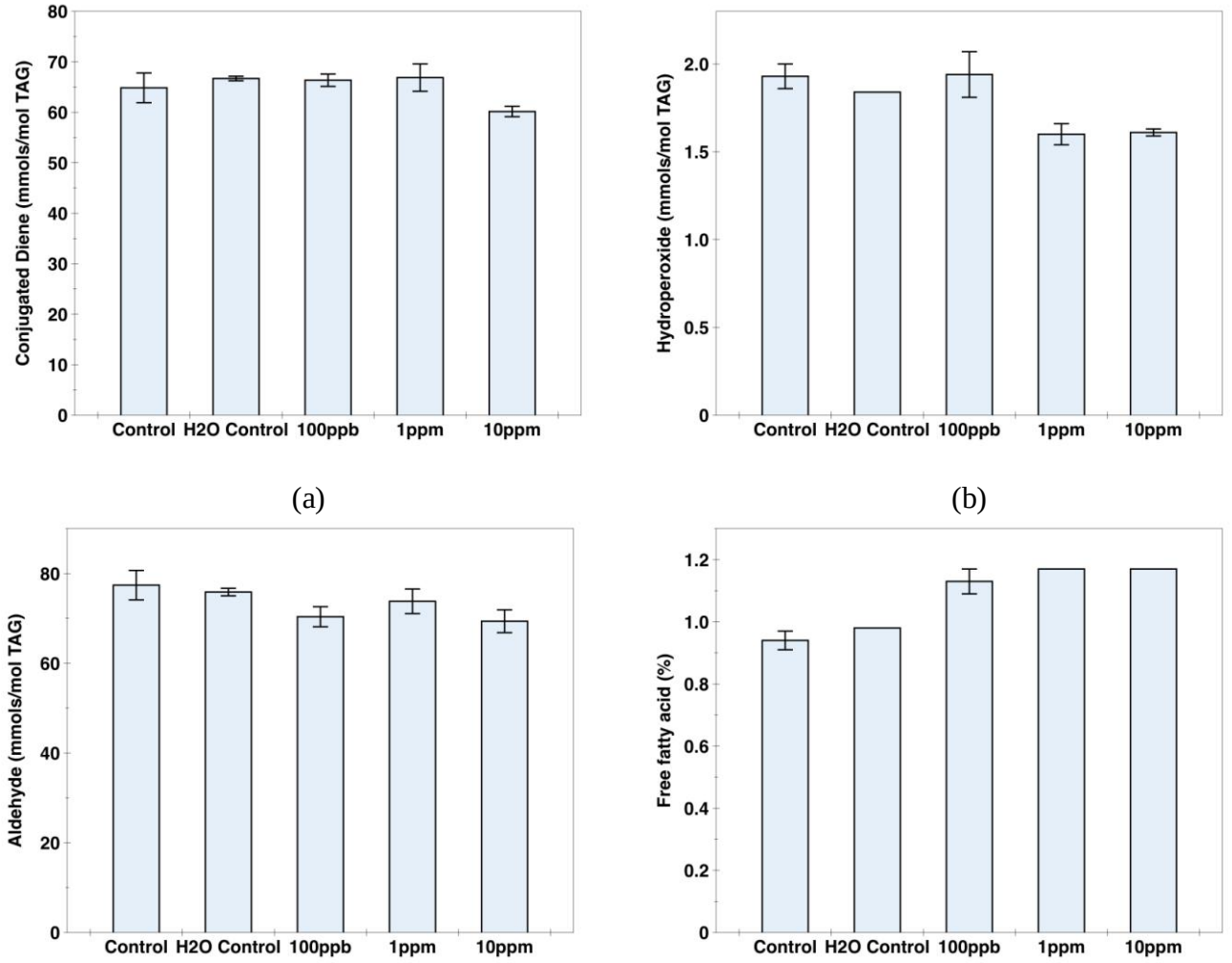


Figure 50. Levels of conjugated dienes (a), hydroperoxides (b), aldehydes (c), and free fatty acids (d) in steady-state oil heated for three hours with 2% water, 0.05% phospholipid, and variable levels of $FeCl_2$.

7. SUMMARY AND INTEGRATION OF DATA

This project investigated the involvement of thermal scissions versus autoxidation (radical chain) mechanisms in degradation of oils at frying temperatures, following oxygen consumption and formation of non-volatile conjugated dienes, hydroperoxides, and aldehydes and integrating these with volatile products collected from the headspace. Results suggest that both mechanisms are active but with different importance and different roles. Thermal scissions provide the main driving force for degradation; their occurrence can be controlled by temperature and their subsequent reactions can be controlled by reduction of oxygen, but they cannot be stopped. Radical chain oxidations are secondary to thermal scissions in both time sequence and importance. They increase the complexity of secondary product distributions and probably increase in importance as heating times are extended. Radical chain oxidations can be eliminated by removal of oxygen. However, while this study has provided a starting point for distinguishing mechanisms active in thermal degradation of oils, it has also shown that reactions are much more complex than previously described. Oxygen consumption that is much greater than products makes it obvious that reactions other than those measured are occurring, so a full accurate picture of thermal degradation processes cannot be generated without more detailed simultaneous analyses, particularly of ketones, lactones, dimers and polymers in the oil.

Results of this study provided strong evidence for thermal scissions in heated oils:

- onset of degradation parallels oil heating directly with no lag period
- degradation occurs even in absence of oxygen

- thermal degradation of hydroperoxides is rapid and continual, resulting in very low levels of hydroperoxides detectable at any given time
- homologous series of volatile short chain alkanes, alkenes, alcohols, and aldehydes not characteristic of radical chain reactions are generated from scissions of acyl chains
- short chain fatty acids are generated from oxidation of short chain aldehydes
- very high levels of both volatile and soluble (core) aldehydes are generated, both on an absolute basis and relative to other products
- multiple classes of products are generated in parallel as soon as heated is applied, rather than sequential development of products as is commonly reported for radical chain oxidation
- initial degradation processes are similar in all oils, while secondary degradations diverge reflecting differences in fatty acid unsaturation
- traditional oxidation catalysts have very low reactivity in heated fresh oils.

In contrast, we saw substantially less evidence for radical chain oxidations initiated by hydrogen abstraction from allylic carbons:

- reactions require oxygen, do not occur when oxygen is excluded
- some delay in onset (production of expected oxidation products)
- presence of conjugated dienes when fatty acids with two or more double bonds are present.
- sequential rather than simultaneous formation of products.

Based on our integrated results we propose the following:

Thermal scissions are the constant *initiator* of oil degradation at high temperatures. As long as heat is applied, thermal scissions actively generate reactive free radicals, although the preferential sites of scission as well as resulting products may change as the oils degrades. Radicals formed in thermal scissions then have several possible fates depending on reaction conditions, degree of agitation of the oil, and the concentration of oxygen molecules close to the radicals (within angstroms).

1) Recombination to reform the original fatty acid – this is probably the dominant reaction in oils being quiescently heated (little swirling or agitation) because there is little force to move the radicals apart.

$$-CH_2-CH_2^{\bullet} + {}^{\bullet}CH_2-CH_2- \rightarrow -CH_2-CH_2-CH_2-CH_2-$$

2) Recombination with other fragments to form new dimer and polymer products, particularly alkanes and ketones of various lengths.

$$-CH_2-CH_2^{\bullet} + {}^{\bullet}CH_2-CH_2-CH_2-CH_2- \rightarrow CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-$$

$$-CH_2-CH_2^{\bullet} + {}^{\bullet}OCH_2-CH_2-CH_2-CH_2- \rightarrow CH_2-CH_2-C(O)-CH_2-CH_2-CH_2-$$

Formation of mixed dimer products becomes more competitive as motion in the oil increases and scission radicals are pushed out of their initial reaction cage; dimers are also enhanced under low oxygen pressures where competition from O_2 reactions is limited.

3) Internal rearrangement of radicals to generate alkenes (favored by mild to moderate agitation and low O_2).

$$-CH_2-CH_2-CH_2^{\bullet} \rightarrow -CH-CH=CH_2 \leftrightarrow -CH=CH-CH_3$$

4) H abstraction from neighboring molecules to form short alkanes (favored by low

O_2 and mild agitation that keeps chains moving, limiting contact between radicals).

$$-CH_2-CH_2^{\bullet} + RH \rightarrow -CH_2-CH_3 + R^{\bullet}$$

5) Reaction with $O^{\bullet}$ formed at high temperatures to generate alkoxyl radicals, $RO^{\bullet}$, that can transform to aldehydes.

$$O\text{-}O \rightarrow 2\,O^{\bullet}$$

$$O^{\bullet} + RCH_2^{\bullet} \rightarrow RCHO + H^{\bullet}$$

Alternatively, the reaction may go through shared addition of oxygen, followed by homolysis of the peroxide:

$$-CH_2-CH_2^{\bullet} + {}^{\bullet}CH_2-CH_2- \xrightarrow{O_2} -CH_2-CH_2OOCH_2-CH_2- \rightarrow CH_2-CH_2O^{\bullet} + {}^{\bullet}OCH_2-CH_2-$$

6) Reaction with O_2 to form terminal peroxyl radicals, $ROO^{\bullet}$.

$$-CH_2-CH_2^{\bullet} + {}^{\bullet}CH_2-CH_2- \xrightarrow{O_2} -CH_2-CH_2OO^{\bullet} + {}^{\bullet}OOCH_2-CH_2-$$

The terminal peroxyl radicals thus formed have multiple options for reaction:

a) **Recombination to form tetroxides**, followed by decomposition to alkoxyl radicals or peroxides, with release of oxygen (well-known Russell mechanism) and alkoxyl radicals or peroxides:

$$-CH_2-CH_2OO^{\bullet} + {}^{\bullet}OOCH_2-CH_2- \rightarrow -CH_2-CH_2OOOOCH_2-CH_2-$$

$$-CH_2-CH_2OOOOCH_2-CH_2- \rightarrow O_2 + -CH_2-CH_2O^{\bullet} + {}^{\bullet}OCH_2-CH_2-$$

$$\text{or} \quad -CH_2-CH_2O\,OCH_2-CH_2-$$

b) **Addition to double bonds to form peroxy dimers**. Terminal peroxyl radicals add to double bonds more readily than mid-chain radicals due to steric considerations.

$$RCH_2OO^{\bullet} + -CH{=}CH_2- \rightarrow \begin{matrix} RCH_2OO \\ | \\ -CH-\overset{\bullet}{C}H- \end{matrix}$$

c) Hydrogen abstraction from neighboring molecules, including allylic carbons of fatty acids, to form hydroperoxides and initiate autoxidation chains.

$$ROO^{\bullet} + LH \rightarrow ROOH + L^{\bullet}$$

Thermal scissions and secondary reactions of the radicals thus formed, with or without oxygen, probably account for most of the initial reaction in heated oils. Formation of peroxyl or alkoxyl radicals (Reactions 6 and 5, respectively) certainly is a major contributor to oxygen consumption from the headspace and generation of Oxipres curves. However, inconsistencies between oxygen consumption and products measured (conjugated dienes, hydroperoxides, aldehydes) indicate that other unaccounted for reactions are most certainly also active. When all products flow to or from oxyl radicals, there is close correlation between oxygen consumption and major oxidation products (at least hydroperoxides and aldehydes, conjugated dienes when H abstraction is active). However, when other pathways are competitive, or when decomposition or stabilization of hydroperoxides is active, obvious relationships are not observed.

Taken all together, our data suggests that thermal scissions occur first when oils are heated and exertoverarching control over the total thermal degradation process. In the presence of oxygen, peroxyl radicals from thermal scissions become the main *initiators* of lipid radical chain oxidation that occurs as a secondary degradation. Thermal scissions during heating cannot be stopped but their consequences can be controlled by limiting oxygen and introducing agitation which facilitates fragmentation and release of small products while reducing dimerization.

An integration of reactions that are consistent with our data is proposed in Figure 51. As depicted on the left side of the scheme, initial thermal scissions generate $R^{\bullet}$

radicals as soon as heat is applied. Alkyl radicals are not effective hydrogen abstractors from allylic hydrogens, but rapidly add O_2 to form terminal peroxyl radicals, $ROO^{\bullet}$, that abstract hydrogens to generate terminal hydroperoxides, ROOH, or recombine to form tetroxides, ROOOOR. Thermal decomposition of both ROOH and ROOOOR releases alkoxyl radicals, $RO^{\bullet}$ that generate aldehydes. This sequence is very rapid at high temperatures, which is why high levels of hydroperoxides and aldehydes are detected immediately upon heating. Assignment of these initial reactions exclusively to thermal scissions is supported by volatile products that are a homologous series of short chain alkanes, alkenes, aldehydes, ketones, and acids, with no evidence of the standard C9,C13 products from autoxidation of linoleic acid [39, 113]. However, detailed analysis of complementary non-volatile products will be necessary to confirm this.

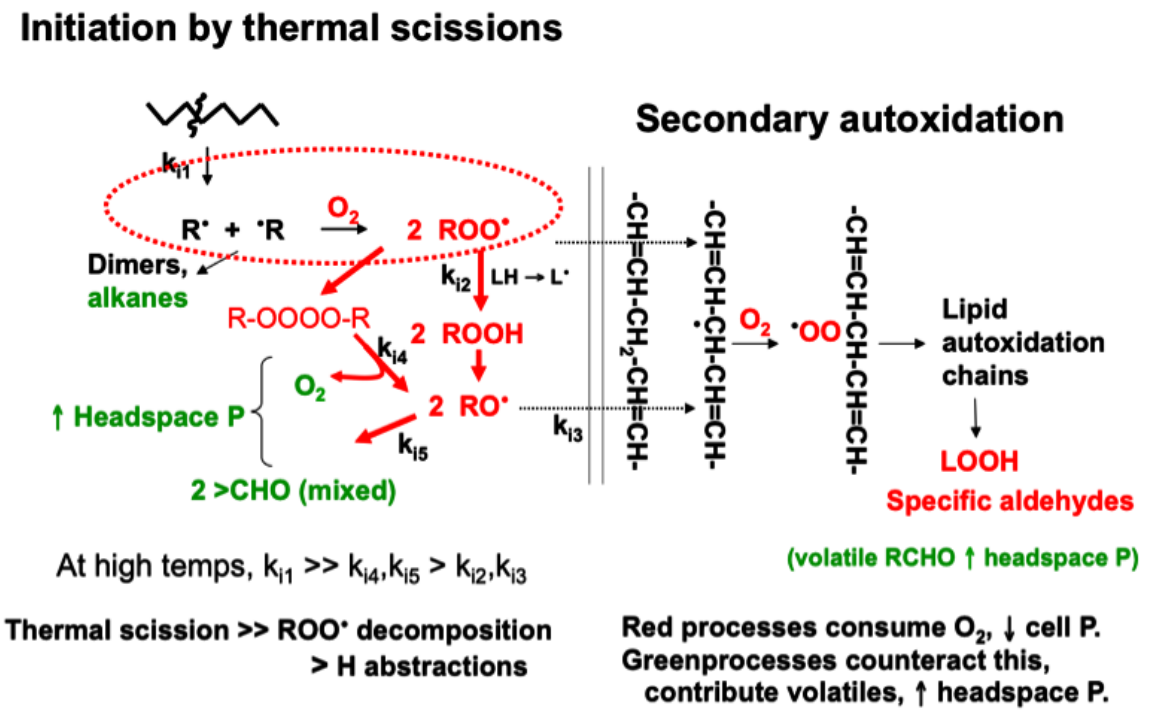


Figure 51. Reaction scheme that accounts for reaction patterns observed in this study and integrates thermal scissions as primary initiators of degradation with autoxidation as a secondary expander of degradation. Most catalytic factors appear to exert their effects on secondary reactions and product distributions where they can be competitive.

The terminal peroxyl and alkoxyl radicals that accumulate in early stages of heating are stronger nucleophiles than alkyl radicals and as such they should readily abstract hydrogens from allylic carbons of unsaturated fatty acids. Hence, once thermal degradation is established, as a secondary process (shown at the intersection and on the right side of Figure 51) these early radicals derived from thermal scissions initiate radical chains that can become self-perpetuating (autoxidation). As heating time is extended then, normal autoxidation processes and product sequences become superimposed on thermal scission processes, and both reaction kinetics and product mixes become increasingly

complex. Based on levels (low) and distribution of volatile products detected (short chain), we judge that reactions occurring in fresh and stripped oils during the three hours of heating in this study were still in early degradation periods. Degradation in the steady-state oil had progressed farther but levels of oxidation products indicated that it was also still in relatively early degradation.

Where do the catalytic factors fit into this process? Data from this study and Repko's parallel analysis of volatile products [113] suggests that factors which are active catalysts of lipid autoxidation at room temperature have little or no effect on kinetics of early degradation during heating of oils because they are not competitive with thermal scissions for initiation. In fresh oil, double bonds are the only targets for chemical catalysts, and the reactions of metals are slow. However, polar degradation products such as hydroperoxides, aldehydes, and carboxylic acids provide foci for molecular interactions such as complexation, hydrogen bonding, and redox reactions. Thus, catalytic factors such as metals, fatty acids, phospholipids, and water are more likely to intervene in secondary and subsequent reactions than initial degradation, and thus alter levels and distributions. of products as heating progresses.

Overall, it was striking how little variation occurred due to or among the different test systems. Of the potentially catalytic or antioxidant factors studied, only metals and water individually were able to modify initial oxidation rates by reducing thermal scission radicals to unreactive ions or by quenching thermal scission radicals, respectively. In the factor combinations, only water plus phosphatidylcholine affected initial oxidation rates, in this case moderately increasing rates. The greater effects were in total oxygen consumption, where most single factors decreased and factor combinations increased

oxygen-consuming secondary processes. Consistent with modifications in secondary degradation pathways were shifts among a variety of products, including products not measured in this study. For all factors, there was also a tendency toward greater divergence of Oxipres curves as heating time was prolonged (data not shown). supporting steady state conditions (Figure 39) as the point where catalytic factors are most important.

A natural corollary of this observation is that metals, water, phospholipids, and free fatty acids may be much more active in batch frying in commercial operations, where oil in vats may be used and reused for several days to weeks, than in industrial frying where the total oil lifetime is usually 8 hours maximum and 3 hours average turnover.

An important observation from the standpoint of quality control was that factors showing little or no effect on oil stability by themselves sometimes became more active when combinations of factors changed solvent and molecular microenvironments so that different chemistry became feasible. For example, iron alone in oil reduces thermal scission radicals to ions, thus slowing initial oxidation rates, and preferentially catalyzes oxygen insertion to form ketones and epoxides rather than peroxides. This would be the dominant effect in fresh oil as it begins heating. When water is present, as occurs after food is added, catalysis shifts back to conventional peroxidation and decomposition of hydroperoxides. When phospholipids are present (from food or poor refining), metal complexes are formed with unknown redox potential and reaction pathways where products other than peroxides, aldehydes, and fatty acids dominate. When fatty acids become available after initial oil degradation (by oxidation or hydrolysis), different

catalytic complexes form, and these can also add aldehydes to further complicate the product picture. Thus, the balance between protection and catalysis may be expected to shift constantly during frying as degradation increases and different products become available for interactions.

Similarly, water may be a pro- or anti- oxidant depending on conditions. Water by itself is primarily protective by hydrogen bonding to hydroperoxides and preventing their decomposition to active alkoxyl radicals. However, when dispersed in oil, water provides localized solvent regions for hydration of metals, phospholipids, and hydroperoxides as well as facilitation of hydrolyses, so when other active molecules are present, water can shift to facilitating oxidation catalysis. Whether the net effect of water will be catalytic or protective will depend on the amount of water and specific types and combinations of catalysts present.

That being said, the lack of notable difference between the various factor combinations was striking. The major effect observed was increase in total oxygen consumption, but the only consistent change was a nominal increase in free fatty acids. Conjugated dienes did not change, showing that the catalytic factors, alone or in combination, did not initiate oxidation chains. The disconnect between increased oxygen consumption and lack of change in standard products suggest strongly that in the heated oils, combinations of catalytic factors acted exclusively on secondary processes, primarily by altering pathways and shifting distributions of products away from standard hydroperoxides, aldehydes, and fatty acids. Volatiles trapped in these studies support this conclusion.

Results of this study show clearly that elimination of oxygen provides the most effective control of oxidation and should be the major focus of any stabilization strategy. The second-most important factor to control (limit) is water, which can be accomplished in part by drying foods before they enter the oil. Metals, phospholipids, and free fatty acids play lesser roles over the short heating times used in this study, but their shifts in oxidation products nevertheless argue for using highly refined oils that have levels of these products reduced to the lowest levels possible (without removing endogenous antioxidants), as well as removing these products regularly (e.g. by filtration or adsorption) from frying oils that are not being replenished frequently.

Degradation in frying oils is conventionally monitored by hydroperoxides, total polars, and total polymers (dimers and above). Observations in this study that combinations of catalytic factors at the very low levels expected to be found in industrial and commercial oils shift products out of standard pathways to as yet unidentified products raises serious questions about how best to analyze degradation in frying oils. Certainly, hydroperoxides, aldehydes, and free fatty acids alone may well give a misleading picture of oil quality.

The low levels of catalysts studies here generated relatively low levels of degradation in the oil and the differences between catalytic factors were small. In terms of oil quality, all oils in this study would have been considered acceptable. However, catalytic processes not competitive with thermal scissions during the short times of heating bulk oils may still drive active oxidation of oil adsorbed to the surface of fried products such as potato chips or corn chips during storage. Thus, perhaps as important as the absolute level of oil degradation is the effect that the small differences in oxidation

products, particularly hydroperoxides and aldehydes (or other products not measured) may have on shelf life of foods fried in the oil. This is a critical issue to follow up in later studies.

10. FUTURE WORK

1. Conduct more definitive studies on degradation processes in heated oils

The two completed studies (this project and the coordinated volatiles analyses) provide very good evidence for thermal scission as the dominant process initiating molecular damage during heating, with autoxidation as a secondary process resulting from reactions of thermal radicals. However, non-volatile products analyzed in this study were limited to class products without detailed identification of their individual components. Thus, judgments had to be made on global total concentrations, and source of generation was not distinguished. For example, terminal hydroperoxides (or aldehydes) formed on thermal scission fragments could not be distinguished from normal hydroperoxides (and aldehydes) formed at allylic carbons in autoxidations, except perhaps they could be induced by timing of development. As discussed in the background section, there are products that form uniquely through thermal scission and cannot be found in autoxidation, and our GC results of a homologous series of volatile alkanes, alkenes and their oxygenated products support this. Nevertheless, due to the pressurized system used in our study, volatiles account for a relatively small portion of all products compared to non-volatiles. Therefore, to validate the reaction sequence we proposed and separate products generated by each pathway, definitive analyses and identification of individual products remaining in the oil must be completed. This will require LC-MS analyses to provide the same kind of product distributions, including alkanes, dimers, and polymers as a function of heating conditions and time. This should also help identify products and pathways not currently considered or anticipated.

2. Measure soluble alkanes, dimers, and polymers

The formation of dimers and polymers are important reactions in high temperature oil degradation. In autoxidation, polymerization occurs only in very late stage and polymers are found only in highly oxidized oil. However, in thermal degradation, heat produces large numbers of radicals immediately so dimerization begins as soon as heat is applied if O_2 is not readily available to divert the radicals to various oxidation products. Catalytic factors such as metals may have contributed to dimerization and polymerization through catalyzing formation of radicals, or they may have acted as antioxidants by converting radicals to ions, thereby preventing combination. Thus, dimers and polymers analysis may provide important verification of catalyst effect. Polymers have practical consequences in that they increasing viscosity and consequently also oil absorption of foods, so understanding extent of development will help guide development of improved stabilization approaches.

This analyses may be accomplished by size exclusion HPLC or MALDI-TOF MS.

3. One reason we did not find expected catalytic effects from metals, fatty acids and phospholipids may be that rapid generation of large quantities of thermal radicals during heating overshadows slower catalysis from these factors. However, results of the present study suggest that these factors may exert their influence during later stages of frying by altering products already produced or by redirecting secondary reactions. For example, metals decompose hydroperoxides and accelerate chain reactions, but the reactions involved are much slower than thermal decomposition of hydroperoxides in early degradations. To test our theory that metals (and other catalytic factors) act primarily in

on secondary products in secondary stages of degradation, oils need to be heated considerably longer (many hours to days) and product shifts followed. In addition, metals and other catalytic factors need to be added to oils that are already more degraded so contain higher concentrations of target molecules. Finally, better methods for incorporating mixed multiple factors into oils need to be developed to determine how factors must come together to be catalytic (or protective). Long time factor studies are not applicable to industrial frying where turnover times are short, but they will be useful for commercial frying where the same oils are being used for days.

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