THERMAL DESORPTION STUDIES OF VOLATILES RELEASED DURING HEATING OF FOOD OILS

by

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ABSTRACT OF THE THESIS

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Lipid degradation occurs extensively during deep fat frying, and controlling it is a great challenge, at least in part because the reaction mechanisms responsible remain controversial. With current trends towards increasing unsaturated fats in frying oils, a more complete understanding of these pathways becomes critical. To gain more detailed information about reactions involved in thermal decomposition kinetics and products of frying oils, corn oil/ high oleic sunflower oil blends (fresh, stripped, and steady-state) were heated at 180°C for three hours in an Oxipres™ oxygen bomb under 2 bars air pressure. Upon release of pressure, the headspace was vented through a Tenax-Carboxen thermal desorption trap to trap volatiles which were then identified and quantitated by gas chromatography-mass spectrometry. Volatile levels and distributions were integrated with oxygen consumption and non-volatile product data from another study to assess relative contributions of thermal scission and autoxidation reaction mechanisms in overall degradation.

Effects of catalytic factors on oil degradation were evaluated by adding metals, phospholipids, fatty acids, and water to the oil blends during heating.

Fresh, stripped, and steady-state oils all generated comparable volatile products, but with differing concentrations and distributions. The main peaks coeluting isopentane/ pentane octane/ hexanal > pentanal/heptanes. Peak analyses revealed homologous series of alkanes, aldehydes, alkenes, ketones, cycloalkanes, and furans were also present. Levels of products generally decreased with chain length. No 2,4-decadienal was detected.

This product pattern and the kinetics of evolution of different products provides strong support for thermal scission as the dominant degradation mechanism that occurs first to generate scission radicals that yield alkanes directly or oxidize to hydroperoxides, aldehydes, and carboxylic acids. Secondary reactions then initiate autoxidation chains. Factors known to have strong effects on lipid oxidation at room temperature appear to influence product distribution rather than degradation kinetics in heated oils.

Integration of volatiles and non-volatiles data with oxygen consumption suggests there are pathways active in thermal degradation that are not being accounted for in current analyses, and these need to be elucidated to fully understand how various factors influence oil degradation as well as to learn how to improve frying oil stabilization.

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My family members and husband, I couldn’t achieve all this without their support.

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1. Introduction

Deep-fat frying is a common cooking method used throughout the world, prized for its relative ease and production of unique tastes and textures that consumers find agreeable. Because frying is used extensively both in industry and domestically, a great deal of research has been focused on the frying oil and the frying process (1-8).

Frying involves complex chemical reactions that continually change from the time the frying oil is first heated to when it has over-extended its usefulness as a medium to cook food. The main reactions that take place are hydrolysis, oxidation, degradation, and polymerization. The triacylglycerols of the frying oil break down as a result of these reactions, which are accelerated by various deleterious factors in the environment and the food being cooked. Antioxidants and chelators have been added to delay the degradation process (8). More common is the formulation of frying oils with some saturated fats to increase oxidative stability (9). However, saturated fats have been attacked for negative health effects in their own right (10) and their use is now also being questioned due to component *trans* fatty acids. Most solid or saturated fats used in frying are hydrogenated, from which *trans*-fatty acids are formed in side reactions (11). Purported links to heart disease have brought *trans*-fatty acids under intense scrutiny by the medical community (10, 12) and even instigated adoption of laws forbidding their presence in marketed (13). Consequently, most foods and even frying oils are increasingly being reformulated with “healthier” polyunsaturated fatty acids (PUFAs).

PUFAs present considerable challenge for stabilization in all foods since they oxidize readily, particularly in frying oils that are heat stressed. Rapid degradation of unsaturated lipids markedly decreases the functional frying time of oils, so new approaches are clearly needed to protect physical, chemical and nutritional quality of high PUFA frying oils.

The most traditional explanation for lipid degradation during frying has been hydrolysis followed by oxidation via a free radical chain reaction (14, 15) First "developed by Farmer and his group at the British Rubber Producers Association" as cited by E.N. Frankel (1980), the autoxidation mechanism involves a simple and straight forward free radical chain reaction:

$$LH \longrightarrow L^{\bullet} + H^{\bullet} \text{ or } H^{+}$$

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

$$LOO^{\bullet} + LH' \longrightarrow LOOH + L'^{\bullet}$$

The Oxidation Theory of thermal degradation holds that oxidative degradation of oils is merely autoxidation accelerated by heat according to Arrhenius kinetics,

$$k = Ae^{-E_a/(RT)}$$

where k is the rate constant of a chemical reaction occurring at temperature T (in deg kelvin), A is the pre- exponential factor related to the frequency of molecular collisions, E_a is the activation energy of the reaction, and R is the Universal gas constant. The Arrhenius equation predicts that reaction rates double for each 10 degrees increase in temperatures. However, autoxidation alone does not account for all of the numerous products that have been identified in frying chemistry.

In the 1970's, Nawar and colleagues proposed an alternative explanation for thermal degradation of lipids (16). They demonstrated that bond scission occurs throughout the triacylglyceride molecule when oil is heated at or above 180°C (deep fat frying temperatures). These scissions then produce a broad range of products, including the homologous series of n-alkanes, n-aldehydes, and short chain carboxylic acids typically seen in lipid degradation volatiles (17). The thermal scission theory further explains the formation of all products, not just ones expected from unsaturated fatty acids (18). However, thermal scission has not been universally accepted in the frying community and remains controversial.

To unravel thermal versus autoxidation reactions involved in thermal degradation of oils and test stabilization approaches, this research group has utilized Oxipres™ oxygen bombs to provide controlled heating of oils under controlled closed atmospheres. Since many pathways are possible and total products are unknown, oxygen consumption by the oils was monitored during heating to track reaction extent independent of specific products (19). To identify the chemistry underlying observed oxygen consumption, non-volatile oxidation products remaining in the oil were also analyzed (20). A strong correlation between oxygen consumed and the degree of oil deterioration was observed (19), but effects of known pro- and anti-oxidants on oxygen consumption were sometimes inconsistent with expected actions (20). Particularly interesting were observations that oxygen consumption with known strong catalysts decreased relative to controls. That the Oxipres oxygen sensor is a pressure transducer not specific for oxygen raised the possibility that volatiles released from degrading

oils added to cell pressures, thus decreasing apparent “oxygen consumption”. Also, additional more detailed product information was needed to supplement and explain product patterns observed in chemical analyses. Both of these issues could be addressed by trapping and analyzing volatile degradation products released from oils during heating in the Oxipres cells.

Therefore, this thesis research investigated volatile release from frying oil heated under controlled conditions in Oxipres™ oxygen bombs to determine both quantities and identities of products generated during heating of oils in the presence of different catalytic factors. Oxygen bomb cells were vented through thermal desorption traps to collect volatiles, which were then desorbed onto a gas chromatography column for analysis. Products were identified by comparisons to standards and by mass spectrometry. Total volatile concentrations were determined to assess potential contribution to (or interference with) apparent oxygen consumption and product distributions were compared to evaluate involvement of thermal scission versus autoxidation mechanisms in thermal degradation of the oil.

2. Literature Review

2.1. The Frying Process

The frying oil is an intricate part of fried food, for without out it the food will not be cooked and the characteristic fried flavor would not be present. The type of oil used for frying is important, as the more saturated the oil the longer it can be used due to its stability (5). The less saturated the oil the more rapidly it degrades and its useful lifetime as a cooking medium is shortened. An understanding of the frying process, therefore, helps focus on the key aspects of how to maintain the quality of the frying oil.

In the deep-fat frying process, the frying oil is heated to 175-200°C. The food is then entirely submerged in the heated oil to be cooked (Figure 1) (21). The food is cooked through the transfer of heat from the oil to the food surface. In the process, the oil goes through a multitude of changes that affect the frying system chemically and physically. These changes include aeration which leads to oxidation and a series of chemical reactions, hydrolysis, polymerization which can lead to an increase in viscosity, adsorption of the oil into the food, and solubilization of food material into the oil.

Aeration effected through the bubbling that occurs when food is added to the oil enhances lipid oxidation and formation of lipid hydroperoxides. The lipid hydroperoxides then breakdown and lead to rapid degradation of the oil through a series of chemical reactions such as scissions, dehydration and free radical formation (22, 23). Release of water from the surface of the food also aerates the oil but also catalyzes hydrolysis of triacylglycerols to release free fatty acids.

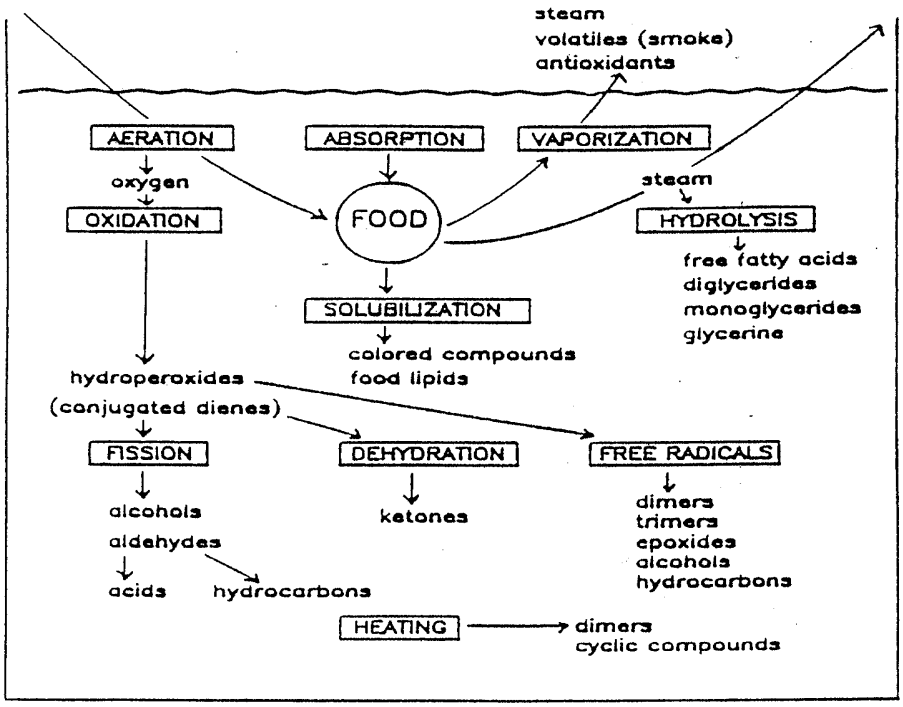


Figure 1. Physical and chemical changes occurring in food and oils during frying. From (21).

Water and air both catalyze thermal decomposition lipids to produce polar products such as epoxides, aldehydes, ketones, acids, mono- and di-glycerides (24). These polar products are important mediators of heat transfer between the oil and food, producing a light thin crust on the food surface that is associated with fried food (25). Flavors associated with fried foods also come from these polar by-products (26).

A unique aspect of the frying process is that some degradation of the oil is needed in order to cook the food properly. The challenge of deep-fat frying is to know when there is an optimum balance of polar by- products in the system. Blumenthal (27) divided frying into five stages defined by frying performance –

break in, fresh, optimum, run-away, degrading (Figure 2, graph). Schaich (28) connected each stage to specific chemical changes (Figure 2, top). The first stage would have too little lipid oxidation while the third stage would have too much lipid decomposition; in both cases the food would come out of the fryer undercooked with an oil saturated surface that is unappetizing and unacceptable to consumers.

There is a small window of time that occurs during the second stage of oil degradation in which the food will be optimally cooked. Many chemical reactions that are important to fried food characteristics occur during this period, but there is also active degradation. Limiting degradation and extending the “optimum” window while enhancing desirable reactions is a significant challenge to frying control. Approaches to accomplishing this include addition of antioxidants to slow the reactions (4, 8, 29), regular addition of some fresh oil to the partially used oil to decrease concentrations of degradation products to balance of polar products (3, 30) and reduce catalysis, and limiting catalysts such as metals that initiate or accelerate lipids autoxidation..

Food has moisture (31), fat and phospholipids, and metals (32) that leach into the frying oil during cooking and act as a lipid oxidation catalysts (33). Food particulates should also be filtered out of the oil to lengthen the quality of the oil (33).

CHEMICAL REACTIONS

	I	II	
Dominant Reactions:	Hydrolysis to 's, mono- di- acylglycerols	Oxidation, scission, some polymerization	Secondary oxidations, scissions, polymerization, cyclization
Major Effects:	1) Increase in surfactant properties 2) Decrease in smoke point 3) Increase in metal chelation (oxidation catalysts) 4) Increase in soap formation	1) Oxidation of unsaturated FA's 2) LOOH decomposition ↓ aldehydes, ketones, lactones, etc. 3) Volatile scission products → odors 4) Non-volatile products → flavors 5) Browning 6) Limited crosslinking	1) Glycerol oxidation to acrolein (sharp odors, toxic) 2) Extensive polymerization → increase in viscosity 3) Increased scissions and oxidation → oil breakdown 4) Formation of toxic products

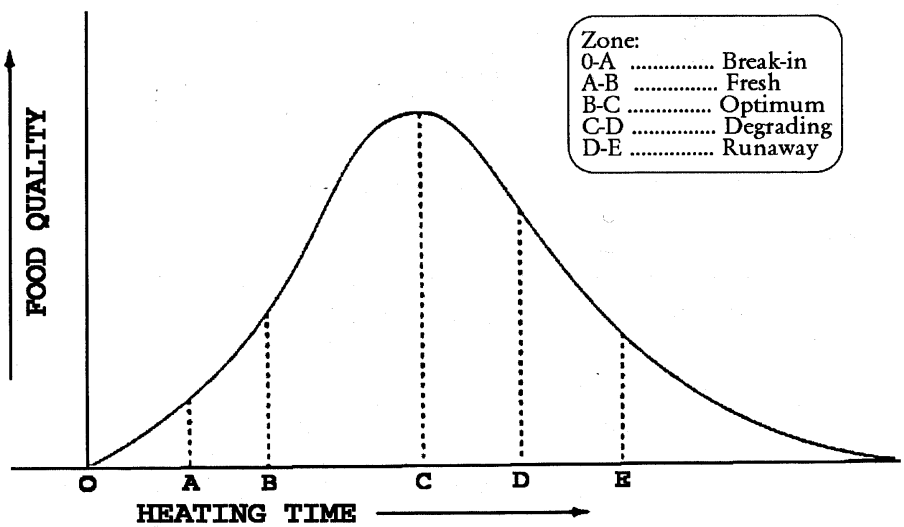


Figure 2. Frying performance curve matched to chemical reactions and physical changes during degradation in frying oils. Adapted from Blumenthal (1991) (27) by Schaich (2008) (28)

2.2. Current Theories of Frying Chemistry

Frying degradation cannot be controlled without understanding the underlying chemistry. Although extensive research has focused on elucidating frying chemistry, considerable controversy still exists regarding details of chemical reactions causing degradation in heated oils.

2.2.1 General: Hydrolysis plus crosslinking and oxidation

Frying oil undergoes a wide variety of chemical transformations during heating. In general, the rate and extent of degradation increases with heating/frying temperature. A diagram of current understanding of degradation processes during frying is shown in Figure 3.

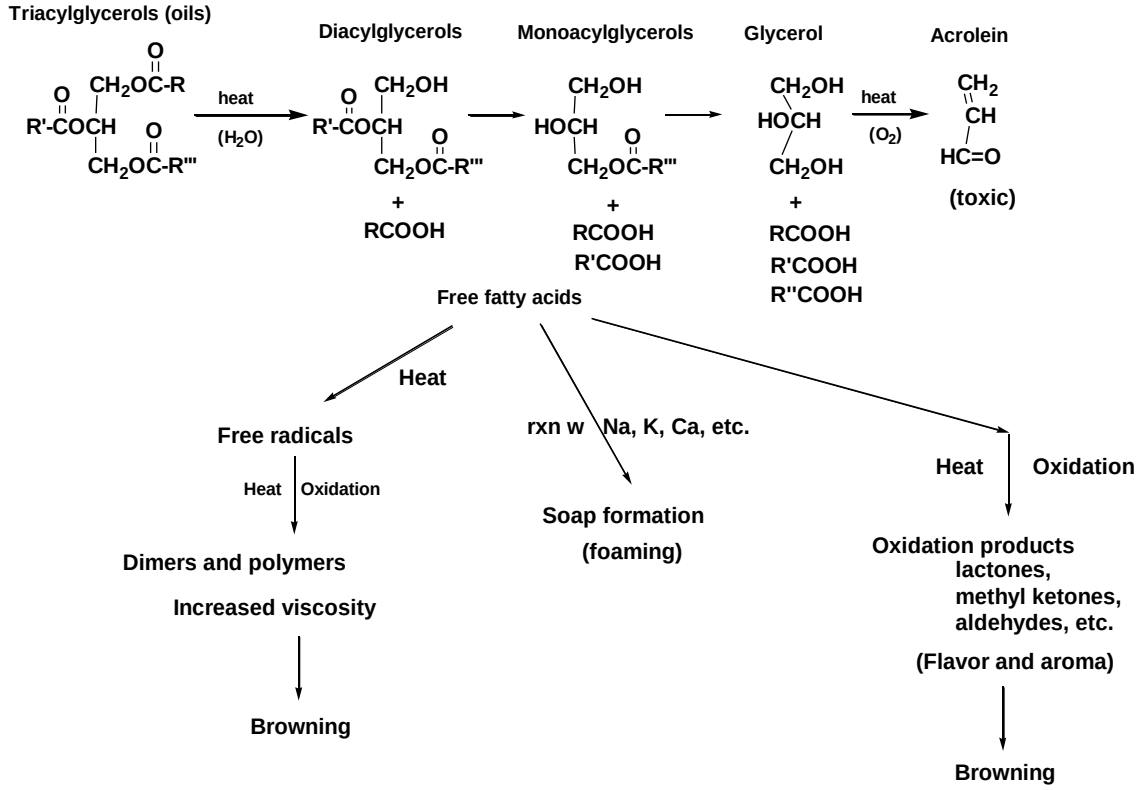


Figure 3. Generalized reaction scheme describing degradation of fats and oils during heating, e.g. during frying (28).

Current thinking describes frying chemistry as involving a series of processes. The first reaction in the presence of food is hydrolysis of triacylglycerides, resulting from heat and moisture introduced by the foods (1). The first products are fatty acids released from their glycerol backbone, plus some mono- and di- glycerides or glycerol (26). Glycerol subsequently oxidizes to acrolein, which has received considerable attention as a potential carcinogen. The reactive free fatty acids then undergo various degradation reactions, including free radical formation, crosslinking, saponification, and oxidation (14). Dimerization and crosslinking are mediated by free radicals and generate products with both carbon and oxygen links, e.g. C-C, C-O-C, C-OO-C (34). Polymerization increases oil viscosity, which in turn, impairs heat transfer to the food surface and increases oil adherence to the food (6). Free fatty acids form soaps with alkali metals or ammonia released from foods (26, 35); soaps contribute to the oil foaming (26) and to off-flavors that develop with extended frying (36). Fatty acids also oxidize to a variety of breakdown products, including the lactones and methyl ketones that are largely responsible for characteristic fried flavors (17, 37), as well as other that contribute to the sharp, acrid, rancid flavors that develop with extended frying (38). Browning reactions involving condensation of carbonyl oxidation products with themselves and with amines released from foods lead to oil darkening that increases with heating time and temperature (39).

The balance between the various thermal products and the specific volatile and non-volatile products generated during frying are determined by the

frying conditions, the fatty acid composition of the oil, and trace contaminants in the oil (25). Frying oil degradation also varies with the type of food being cooked, the total frying time, whether the oil is being heated continuously or intermittently (40), the moisture content of the food (25), and the fat content and composition of the food, since that fat leaches into the frying oil and both will breaks down and contributes catalysts into the frying oil (26).

Degradation reaction in frying oils are important not only for the quality of food being fried (e.g. chicken, French fries, donuts) (35), but also for the stability of fried foods being stored (e.g. potato chips and fried snacks) because the oil adsorbed during frying is a critical determinant of subsequent degradation (41-43).

While the process described in Figure 3 provides a picture that can explain general observed behaviors of frying oils during heating, this presentation deals only superficially with the chemistry underlying the reactions listed. For many years, these reactions remained of mostly academic interest since the food industry could largely control frying by mixing some saturated fats with the frying oil (44). However, recent reformulations of foods and frying oils with higher polyunsaturated oils for health have posed significant technical challenges and are forcing food companies to rethink approaches to food formulations and stabilization. Traditional solutions for stabilizing foods and frying oils have not been successful with these reformulations. New information about lipid oxidation reactions in general, and frying degradation in particular, is therefore needed, in order to develop more effective approaches for stabilizing frying oils and the

foods fried in them. Consequently, issues formerly considered merely theoretical have now assumed critical practical importance in that insights gained from elucidating detailed chemical mechanisms can be harnessed to control degradation. To provide a basis for understanding the controversy and its implications, reviews of the two chemical theories of thermal degradation that provide the driving force for this thesis research are presented below.

2.2.2 Theory 1: Thermal degradation results from thermal scission of bonds in lipids

Nawar and his students first proposed the theory of thermal scission of the triacylglycerols or TAG's, with their research being conducted mostly in the 1970's. They discovered that the high temperatures of frying have enough energy to break bonds at all points of the TAG. The same scission patterns were found to occur under nitrogen and air, demonstrating that the degradation was independent of oxidation and related only to the thermal energy (45).

Nawar and colleagues demonstrated that thermal cleavages occurred in specific positions, generating four classes of decomposition products (Figure 4) (16). When scission occurs between the glycerol backbone and the ester oxygen, free fatty acids and acrolein (A) are formed. When the scission occurs between various methylene groups along the acyl chain, a homologous series of hydrocarbons (B) are generated. Propene- and propane-diol esters (C) are produced by scission between the carbons of the glycerol backbone. The last class of products, mono- and di- acylglycerols (D) are created by scission between the oxygen and the carbonyl of the ester function. Choe and Min (6)

proposed that the various strengths of hydrogen-carbon bond of fatty acids accounted for the differences of oxidation rates of stearic, oleic, linoleic, and linolenic acids and dominant scission positions during thermal oxidation. Hydrocarbons and derivative products generated from attack on the acyl chain (B) are volatile and would collect in the head space of the oxygen bomb's closed chamber; therefore, these were the focus for assessing

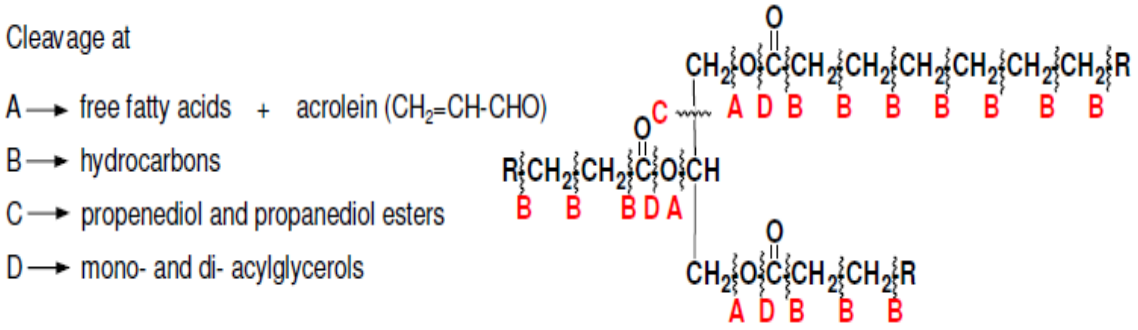


Figure 4. Patterns of thermal scissions occurring in heated triacylglycerols. Products are those occurring in the absence of oxygen. Redrawn from (16).

active mechanisms in this study. Degradation products centered around the glycerol backbone (classes A, C, and D) are too large to be volatile so they remain in the oil as non-volatiles.

Both thermal scission and autoxidation can generate some of the same end products but the pathways in which they are formed may be different (46). During thermal scission, thermal energy disrupts C-C bonds at many positions on the acyl chain (Figure 5), producing two free radicals per scission (45). The free radicals that are formed either generate products or can add oxygen to form terminal peroxyl radicals. The latter can initiate lipid autoxidation by abstracting

hydrogens from allylic positions on acyl chains. In contrast, autoxidation requires catalysts such as metal, light or singlet oxygen to initiate the reaction (47), and attack on the acyl chain occurs only at allylic carbons (next to double bonds). Consequently, decomposition products generated by the two processes should differ.

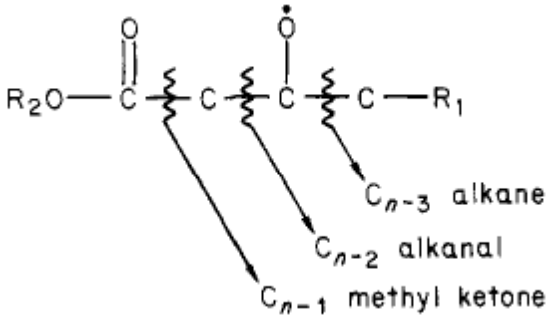


Figure 5. Cleavage scission of alkoxy radical at the β-carbon produces methyl ketones, scission between α- and β- carbons produce alkanal, and scission between the β and γ-carbons produce alkanes (17).

Since heat is the major driving force in frying, effects of heat lipid degradation cannot be ignored. Higher temperatures and longer frying times increase the amount of degradation occurs (27). Thermal reactions mediated by heat include dehydration, decarboxylation, double bond conjugation, polymerization, and carbon-to-carbon cleavages (45). Heat is a constant force during frying, while initiation and propagation stages of autoxidation can both be delayed by the addition of antioxidants (8). Thermal scissions thus become a critical point of interest in understanding the complexity of thermal degradation of lipids. Nawar (16) has shown that the breaking of the lipid chains at all points

leads into the formation of aldehydes, ketones, and homologous series of alkanes (radical recombination products).

The high levels of free radicals are formed by thermal scissions of lipids rapidly deplete antioxidant in frying oils (48, 49).

2.2.3. Theory 2: Thermal degradation results from lipid autoxidation thermally accelerated according to Arrhenius kinetics

Autoxidation is a free radical chain mechanism that requires initiators such as such as metal, light, and heat, but once started is self-propagating (7). The free radical chain reaction consists of three stages of development: initiation, propagation, and termination (Figure 6).

Initiation: The initiation stage of autoxidation involves the formation of the first free radical (referred to as the *ab initio* radical) by various initiators such as metals, light, heat, or other radicals (Rx. 1, Figure 6), followed by rapid addition of oxygen to the radical to form a peroxyl radical (Rx. 2, Figure 6) (50). Formation of free radicals starts the chain reaction of lipid oxidation, while the driving force behind the chain reaction is hydrogen abstraction by the free radicals to transfer radicals to new lipids and thereby propagate the chain. Monitoring oxidation reactions by oxygen consumption measures Reaction 2.

Initiation is difficult to control due to the multiple ways in which a free radical can be created. Ways to control initiation are to reduce exposure to heat and light and remove metals by chelation.

CLASSICAL FREE RADICAL CHAIN REACTION MECHANISM OF LIPID OXIDATION

Initiation ***(formation of ab initio lipid free radical)***

$$L_1H \xrightarrow{k_i} L_1^{\bullet} \quad (1)$$

Propagation

Free radical chain reaction established

$$L_1^{\bullet} + O_2 \underset{k_\beta}{\overset{k_o}{\rightleftharpoons}} L_1OO^{\bullet} \quad (2)$$

$$L_1OO^{\bullet} + L_2H \xrightarrow{k_{p1}} L_1OOH + L_2^{\bullet} \quad (3)$$

$$L_2OO^{\bullet} + L_3H \xrightarrow{k_{p1}} L_2OOH + L_3^{\bullet} \text{ etc.} \dashrightarrow L_nOOH \quad (4)$$

Free radical chain branching (initiation of new chains)

$$L_nOOH \longrightarrow L_nO^{\bullet} + OH^{-} \text{ (reducing metals)} \quad (5)$$

$$L_nOOH \xrightarrow{k_{d1}} L_nOO^{\bullet} + H^{+} \text{ (oxidizing metals)} \quad (6)$$

$$L_nOOH \xrightarrow{k_{d2}} L_nO^{\bullet} + {}^{\bullet}OH \text{ (heat and uv)} \quad (7)$$

$$\left.\begin{matrix} L_nO^{\bullet} \\ L_nOO^{\bullet} \\ HO^{\bullet} \end{matrix}\right\} + L_4H \xrightarrow[k_{p1}\ k_{p3}]{k_{p2}} \left.\begin{matrix} L_nOH \\ L_nOOH \\ HOH \end{matrix}\right\} + L_4^{\bullet} \quad \begin{matrix}(8a)\\(8b)\\(8c)\end{matrix}$$

$$L_1OO^{\bullet} + L_nOOH \xrightarrow{k_{p4}} L_1OOH + L_nOO^{\bullet} \quad (9)$$

$$L_1O^{\bullet} + L_nOOH \xrightarrow{k_{p5}} L_1OH + L_nOO^{\bullet} \quad (10)$$

Termination ***(formation of non-radical products)***

$$\left.\begin{matrix} L_n^{\bullet} \\ L_nO^{\bullet} \\ L_nOO^{\bullet} \end{matrix}\right\} + \left.\begin{matrix} L_n^{\bullet} \\ L_nO^{\bullet} \\ L_nOO^{\bullet} \end{matrix}\right\} \xrightarrow[k_{t2}\ k_{t3}]{k_{t1}} \text{polymers, non-radical monomer product (ketones, ethers, alkanes, aldehydes, etc.)} \quad \begin{matrix}(11a)\\(11b)\\(11c)\end{matrix}$$

Radical scissions

$$\left.\begin{matrix} LOO^{\bullet} \\ LO^{\bullet} \end{matrix}\right\} \xrightarrow[k_{ts2}]{k_{ts1}} \text{non-radical products (aldehydes, ketones, alcohols, alkanes, etc.)} \quad \begin{matrix}(12a)\\(12b)\end{matrix}$$

i - initiation; o-oxygenation; β-O_2 scission; p-propagation; d-dissociation; t-termination; ts-termination/scission

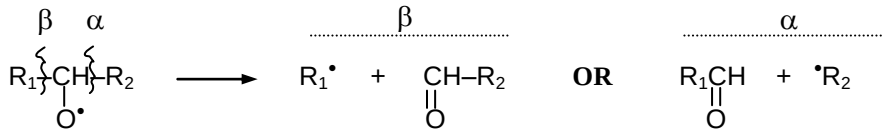
Figure 6. Free radical chain reactions in lipid autoxidation. From (20), used with permission.

Propagation: Propagation is the second stage of autoxidation in which the free radical chain reaction is established. As oxidation starts, lipid peroxyl radicals abstract hydrogens from adjacent lipid molecules to form hydroperoxides and leave behind new radicals (Rxs 3 and 4, Figure 6). The hydroperoxides decompose to alkoxyl radicals (Rx 5, Figure 6) and with heat or uv light also hydroxyl radicals (Rx 7, Figure 6), which then similarly abstract hydrogens to form lipid alcohols and leave behind new radicals. Unlike thermal scissions which occur at multiple positions on both saturated and unsaturated fatty acids, hydrogen abstraction by lipid peroxyl and alkoxyl radicals occurs only at allylic or doubly allylic positions of unsaturated lipids, where C-H bond energy is sufficiently weak. Propagation results in a buildup of the hydroperoxides (50).

Radical abstraction does not result in breakage of double bonds. Rather, the double bond migrates, forming conjugated dienes when two or more double bonds are present. Conjugated dienes can be measured by their absorbance at 234 nm (50).

Termination: Individual radicals (not total radical chains) are terminated when they react to form non-radical products without leaving behind a new propagating radical. This occurs when radicals and hydroperoxides accumulate to high enough levels that interactions occur and generate dimers dimers (Rx. 11, Figure 6) (47), and when alkoxyl radicals undergo scission to generate aldehydes (Rx. 12, Figure 6). Scission products, in particular, provide markers characteristic of autoxidation since peroxyl and later alkoxyl radicals form preferentially at H abstraction positions, i.e. the outermost carbons of the double

bond system. For linolenic acid, these positions are C-9 and C-13, while for linolenic acid, they are C-19 and C-16. Scission can occur at the C-C bond on either side of the alkoxyl radical C-O bond, generating two [different] fragments in each case:



α-Scission occurs on the side closest to the –COOH head group, and β scission occurs on the side closest to the $–CH_3$ terminus. The radicals in scission of alkoxyl radicals can add oxygen to form peroxyl radicals and continue the chain, can abstract hydrogens directly to form alkanes, can combine with other radicals to form various products, or can internally rearrange to alkenes. Hexanal from α-scission of C13-O• and 2,4-decadienal from β-scission of C9-O• in linolenic acid are probably the two most typical products associated with lipid oxidation (47).

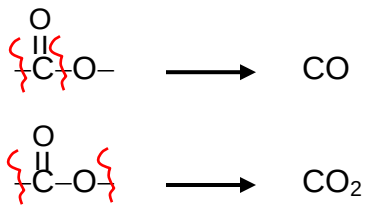
2.3. Comparisons of products expected from thermal scissions versus autoxidation of lipids

As discussed previously, in thermal degradation of oils, such as occurs during frying, there are many chemical reactions occurring simultaneously. The question arises, what are the roles of thermal scission and autoxidation mechanisms? Is only one active or strongly dominant as argued in current theories, or are they somehow integrated to form a hybrid mechanism in thermal degradation of lipids? According to the currently most prevalent theory, thermal

degradation results from autoxidation reactions accelerated by heat according to the Arrhenius equation (see Section 2.3), i.e. reaction rate doubling for every ten degrees in temperature. However, the assumption in the Arrhenius equation is that a single reaction is involved and that mechanisms do not change. That is not the case with lipid oxidation which is a mixture of reactions, all of which are affected differently by heat. The Arrhenius equation may hold for moderate increases in temperatures, but it cannot accurately predict rates when mechanisms change or factors other than temperature affect the rates (11). Reaction mechanisms for lipid autoxidation change at several temperatures, starting at just above 40°C where hydroperoxides begin decomposing (Schaich laboratory, unpublished data), and several changes occur again at higher temperatures, particularly between 150 and 180°C (51). Therefore, accelerated autoxidation alone cannot explain thermal degradation of lipids, and a more complete and accurate explanation is needed.

2.3.1 Products from thermal scission

Thermal scission products occur in both saturated and unsaturated fatty acids producing a broad range of non-oxidative and oxidative decomposition products, depending on conditions. Under limited oxygen, saturated acids produce a homologous series of normal alkanes and 1-alkenes from scissions along the acyl chain (n-alkanes are found in greater quantity than their 1-alkene counterparts (52)), fatty acids and acylglycerols from scission at the ester bond, and acrolein from glycerol; CO and CO_2 are released via scissions within and around the carboxyl group (37).



In unsaturated fatty acids, scissions occur preferentially close to the double bond, releasing the corresponding alkanes and alkenes. These radicals are also prone to dimerization by recombination and addition to double bonds in the same or different triacylglycerols (52). Diels-Alder dimers are unique to thermal degradation. They arise from reaction of isolated double bonds with conjugated double bonds (53). Other intra- and inter- molecular cyclization products can be formed by several different means giving rise to trimers as well as cyclic monomers (37).

In the presence of oxygen, products become complex mixtures of scission products and secondary autoxidation products. Thermal scissions still occur and generate fragmentation products, but initial scission radicals also add oxygen to form terminal peroxyl radicals that either decompose to corresponding short-chain aldehydes, add to double bonds, or abstract hydrogens to form terminal (16, 37, 45, 53). The latter reaction initiates autoxidation reactions. Since scissions occur preferentially at positions α, β, and γ to double bonds, some products common to autoxidation are shared. For example, pentane is generated by scission β to C13 in double bonds or alkoxyl radicals. Pentane was the most abundant product observed in autoxidizing linoleic acid (52), or (54) and was also the dominant product in corn oil heated to 180°C (51).

The small soluble and volatile products that contribute to characteristic flavors and odors of fried foods arise from secondary oxidations of radicals in both reactions. For example, methyl ketones and lactones contribute to characteristic fried flavors results from decomposition of secondary products. Hydroxy-fatty acids formed by decomposition of internal hydroperoxides add to the acid carbonyl to yield lactones (45); methyl ketones are generated via scissions two carbons before alkoxyl radicals (45).

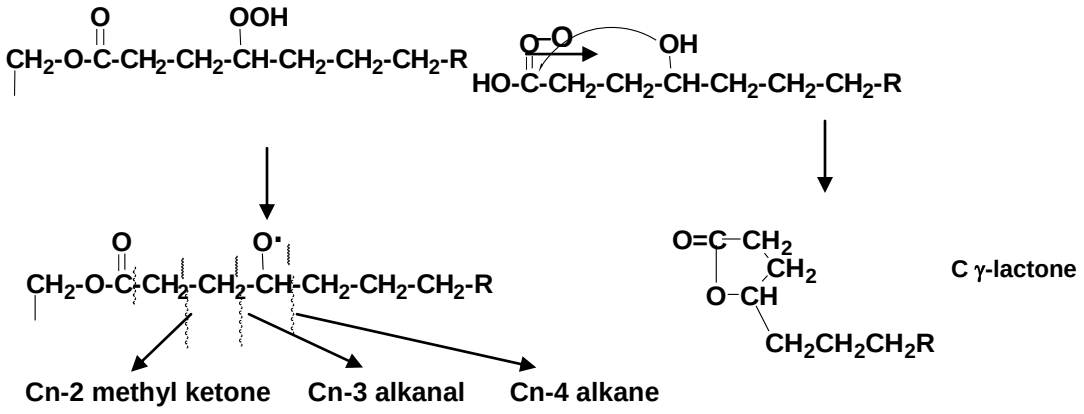


Table 1. Expected Thermal Scission Products (17, 37, 46)

Expected Thermal Scission Products				
Alkanes	**Aldehydes**	**Alkenes**	**Ketones**	**Lactones**
butane			me-ketones	y- lactones
pentane				
	hexanal			
heptane				
octane	octanal	2-octene		
	2- octenal			
nonane	nonanal			
decane				
undecane				
dodecane				

2.3.2 Products from autoxidation

Three different types of products are formed during oxidation: primary, secondary and tertiary. The major initial lipid oxidation product is lipid hydroperoxides, which are hard to track and quantify during heating because they decompose so rapidly at elevated frying temperatures. Hydroperoxides form at allylic carbons (next to double bonds) and predictable products characteristic of autoxidation arise from these positions. In oleic acid which has one double bond (C9-C10), dominant products arise from C8-, 9-, 10-, and 11-hydroperoxides (Table 2). Linoleic acid with two double bonds has products arising from C 9-, 11-, and 13- hydroperoxides and scissions occurring within two carbons of these positions (Table 3).

Hydroperoxides degrade rapidly at elevated temperatures through the fission of the hydroperoxide O-O bond, producing an alkoxyl radical plus a hydroxyl radical, both of which have a number of sybsequent reaction pathways

possible (47). Internal rearrangements generate epoxides, hydrogen abstractions yield alcohols, and scissions on either side of the alkoxyl carbon produce characteristic aldehydes, alkenes, and other carbonyls (47, 55). At high temperatures, rapid oxidation of aldehydes generates short chain carboxylic acids, which can be distinguished easily from the long chain acids resulting from hydrolysis of triacylglycerols in oils. The distribution of products observed in a given oil is determined by the peroxide location on the fatty acid (56) as well as conditions of reaction. Each oil contains differing amounts of fatty acids, with each fatty acid having certain decomposition patterns that produce larger amounts of specific volatiles. The major volatile products for oleic acid include: nonanal, octanal, undec-2-enal, undecanal, heptane, octane and 2-decenal (57) see table 4. The major volatile products for linoleic acid include hexanal, 2,4 decadienal, 2-octenal, and 2-heptenal (46, 47) see Table 3. The major volatile products from both oleic and linoleic fatty acids are saturated and unsaturated aldehydes and saturated alkanes.

Table 2. Products commonly generated by autoxidation of oleic acid. Most prevalent products are designated in bold font. Data compiled from (58, 59).

Hydroperoxide position

C8		**C9**	
α	β	α	β
2-undecenal	8-oxo-octanoic acid	**2-decenal**	9-oxo-nonanoic acid
undecanal	decanal	8-oxo-octanoic acid	nonenol
heptanoic acid	1-decene	octanoic acid	1-nonene
7-oxo-heptanoic acid	nonanol	8-HO-octanoic acid	**nonanal**
7-HO-heptanoic acid	nonane	heptanoic acid	**octanal**
hexanoic acid	**nonanal**	7-oxo-heptanoic acid	
6-oxo-hexanoic acid			
6-HO-hexanoic acid			
formaldehyde	formaldehyde	formaldehyde	formaldehyde
C10		**C11**	
α	β	α	β
nonanal	10-oxo-8-decenoic acid	9-decenoic acid	11-oxo-9-undecenoic acid
9-oxo-nonanoic acid	octanol ?	**octanal**	heptanol
8-nonenoic acid	octane	10-oxo-decanoic acid	heptane
octanol	**octanal**	nonanol	heptanal
octane	heptanol	nonane	hexanol
octanal	heptane	**nonanal**	hexane
formaldehyde	heptanal	formaldehyde	hexanal
	formaldehyde		formaldehyde

Table 3. Products commonly generated in oxidizing linoleic acid. Most prevalent products are designated in bold font. Data compiled from (59).

Hydroperoxide position

C9		**C13**	
α	β	α	β
8-oxo-octanoic acid	9-oxo-nonanoic acid	12-oxo-9-dodecenoic acid	13-oxo-9,11-tridecadienoic acid
2,4-decadienal	1,3 nonadiene	**hexanal**	**pentane** pentanol
Octanoic acid	3-nonenal	9,11-dodecadienoic acid	
8-HO-octanoic acid	1-HO-2,4-nonadienol pentanal	11-HO-9-undecenoic acid	
Heptanoic acid		9-undecenoic acid	butanol
Pentyl furan		11-oxo-9-undecenoic acid	
	butane		
		formaldehyde	butanal formaldehyde
C11			
α	β		
3-Octenal	**2-Heptenal**		

Tertiary products result when secondary products degrade further or polymerize with each other or with the free fatty acids still in the oil, leading to changes in physical characteristics of heat-abused oils (60). Polymerization involves primarily highly unsaturated non- volatile molecular segments in the oil, and often occurs in preference to forming polar by-products (61). Polymerization is particularly favored when there is insufficient oxygen in the system to create hydroperoxides (62). In this case, carbon-to-carbon or carbon-to-oxygen-to-carbon bridges form between fatty acids, creating dimers, trimers, and higher polymers with both cyclic and acyclic products (34) (Figure 7). The specific types

of dimers and polymers dominating in any given system depend on the type of oil, temperature, and the length and pattern (continuous or intermittent) of frying times. Polymer products can become very large and eventually increase the viscosity of the oil and contribute to browning (6).

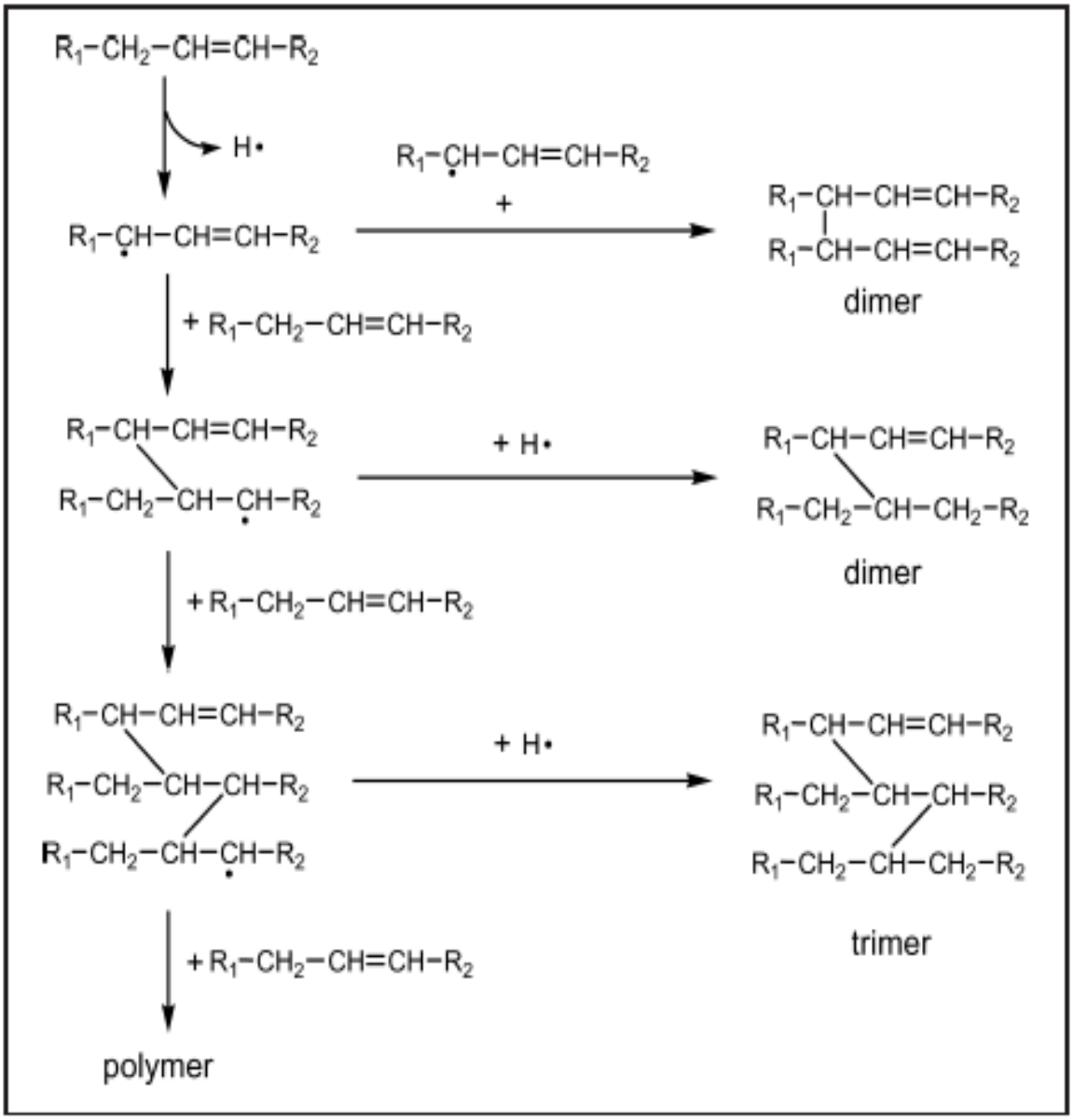


Figure 7. Acyclic polymer formation from oleic acid during deep fat frying (6).

2.4. Factors catalyzing or accelerating lipid degradation

2.4.1 Heat

Heat is one of the main factors accelerating degradation of oils. At moderately elevated temperatures, the major effect of heat is to decompose hydroperoxides to more reactive alkoxyl and hydroxyl radicals that then accelerate autoxidation (63). At higher temperatures, rates and complexity of degradation increase with temperature as activation energies of additional reactions are overcome. These effects are especially critical in deep fat frying systems where the average frying temperature is 180°C. The increased energy in the system weakens the bond strengths of the triacylglycerides so that bond scission occurs more readily, creating various decomposition products (37). Obviously, degradation increases with heating time. Also, intermittent heating is more detrimental to the degradation process (40) because secondary chains become established and hydroperoxides accumulate while the oil is cool.

2.4.2 Oxygen

Oxygen plays an important role in lipid degradation because, obviously, it is the active oxidizing agent, and because it converts non-reactive or slowly reactive species such as alkyl radicals to reactive agents such as peroxyl and alkoxyl radicals. That being said, the rate of oxidation is oxygen-dependent only at very low oxygen levels, i.e. when fewer oxygen molecules than initiating radicals are present. This level varies with the reaction system but typically is 1% or less oxygen. As long as sufficient oxygen is present to add to every initiating $L^{\bullet}$

present, oxidation rates are dependent on initiation processes rather than oxygen.

Oxygen plays additional roles in thermal degradation and frying, converting thermal scission processes to autoxidation chains and generating polar products that are surfactant and increase contact of oil with foods. The latter is necessary for efficient heat transfer to foods (27, 64).

2.4.3 Transition Metals

Metals are perhaps the single-most active chemical initiators and catalysts of lipid degradation. The two transition metals found most often in food products are copper and iron (62), but other transition metals such as nickel and cobalt, obtained as contamination from food processing equipment, are also active catalysts. Metals, particularly iron from meats, also leach into the frying oil from foods being fried and increase the rate of lipid decomposition (62).

By both donating and accepting electrons, transition metals have the capability of both initiating lipid oxidation (generating the *ab initio* radical) and accelerating secondary reactions (65). Transition metals can initiate lipid oxidation by direct electron transfer to a lipid, breaking a double bond to create an *ab* initio radical that starts free radical chains and becomes lost in the process, or by indirect transfer via intermediate formation of metal-oxygen complexes (47). Transition metals catalyze lipid oxidation by decomposing hydroperoxides to radicals. Reduction of hydroperoxides to alkoxyl radicals is much faster and more common than oxidation of hydroperoxides to peroxyl radicals (65). Although metals are very efficient catalysts in most foods and

biological systems and accelerating effects on frying degradation have been reported (62), their reactions are very slow compared to thermal scissions during frying, so the mechanisms by which they affect stability of oils during frying remains unclear and controversial.

It is important to note several unique effects of metals that may be relevant in frying (47, 65). First, frying is done in neat oils that pick up some moisture during cooking but remain very hydrophobic reaction environments. Under such conditions, metal catalysis mechanisms and products are very different than in aqueous systems. For example, in aprotic media, metals such as iron catalyze oxygen insertion to form epoxides and ketones in high yields rather than initiating radicals and decomposing hydroperoxides (66). Formation of hypervalent iron (e.g. ferry iron) complexes with hydroperoxides is also facilitated, and the result is very rapid conversion of hydroperoxides to alcohols and epoxides (67). The result is a major shift in products, which may never be detected if only conventional oxidation products are monitored (47, 65).

It is also important to note that metals may paradoxically become antioxidants at higher concentrations when they convert radicals to ions (47, 65)

Since metals are known to cause many problems in stability of oils before, during, and after frying, chelators such as citric acid are commonly added to frying oils at up to 100 ppm for stabilization (68).

2.4.4 Free Fatty Acids

Free fatty acids are released from triacylglycerols via scission of the ester bond with glycerol, usually through hydrolysis (69). Unsaturation of the fatty acids increases the rate of hydrolysis (45). Free carboxyl groups are very reactive and open potential for a number of competing effects.

Free fatty acids are amphiphilic (contain both hydrophilic and hydrophobic groups) so are surface-active and decrease surface tension in the oil. The decrease in surface tension then increases the rate of diffusion of oxygen into the oil, enhancing oxidative degradation (70).

Free fatty acids contribute characteristic fried flavor and aroma as they break down further into volatile products. However, whether free fatty acids increase or decrease oxidation rates remains controversial. Both faster oxidation (6) and slower oxidation of free acids has been reported. Apparent enhancement of oxidation may occur when acid functions –COOH hydrogen bond to hydroperoxides and slow their decomposition. Inhibition of oxidation by free fatty acids may result from non-radical decomposition of hydroperoxides by carboxylic acids (71).

Free fatty acids complex with soft metal ions to form alkaline containment material or soaps (35). Soaps produce expected off-flavors, but also increase foaming by decreasing the interfacial tension of the frying oil. Fatty acids also polymerize with other secondary products, such as mono and di-glycerides. This increases the viscosity of the frying oil and also contributes substantially to decreases in smoke point (6).

2.4.5 Phospholipids

Phospholipids such as phosphatidalycholine and phosphatidyl-ethanolamine are naturally found in crude extracts of vegetable oils. Their actions in oil remain poorly understood and have many contradictory reports, perhaps because they have many potential reaction mechanisms and so their effects vary with the food system and application. At low levels (3 – 60 ppm), phospholipids inhibit oxidation by chelating metals, specifically iron (70) and by non-radical decomposition of hydroperoxides (71). In contrast, at low metal contents, phospholipids may be pro-oxidants in purified oils by altering the nature of the oil interface, decreasing surface tension, and increasing diffusion of oxygen into the oil (35, 70).

2.4.6 Light

Photosensitizers are removed from frying oils during refining, so only ultraviolet light is a potential problem. Ultraviolet wavelengths greater than ~200 nm do have enough energy to break bonds and form free radicals; rather, uv light most often just induces ionization, which is a slow process (47). The point at which uv light has the greatest effect on lipid oxidation is in accelerated homolytic scission of the O-O bond in hydroperoxides to release reactive alkoxyl and hydroxyl radicals that carry on hydrogen abstraction at faster rates, thus accelerating lipid oxidation (47).

Direct light effects occur in home or commercial frying where fryers are open and exposed to light. Industrial fryers are mostly covered, so light effects

occur predominantly in products on conveyor belts after removal from the fryer, as well as in packages if light filters are not included.

2.5. Approaches to stabilizing thermal degradation

Stabilizing lipid oxidation is key to increasing the usefulness of the frying oil. Many factors have been implemented over time to increase and maintain the window of optimum frying conditions, including replenishment of the oil used with fresh oil, the use of saturated fats, the exclusion of oxygen, the addition of antioxidants, and the controlling of the catalysts (6, 23). Only an overview will be presented here.

2.5.1 Exclusion of oxygen

The main catalyst to limit is the amount of oxygen in contact with and dissolved in the oil. This is not an easy task in open systems where oxygen is always available. Major approaches to reduce oxygen exposure in the food industry include

- maintaining oils under nitrogen during all handling, transport, and storage
- creating a thin layer of nitrogen across the surface of the oil during heating
- utilizing a “blanket of steam,” or micature layer formed by vaporization of water released from foods during the frying (46, 69).
- Using covered rather than open vats.

2.5.2 Saturated versus unsaturated fatty acids

The stability of the frying oil is a huge problem for the food industry. Previously, saturated (usually hydrogenated) and mono-unsaturated oils were used for oxidative stability because the higher the saturation of oil, the higher the thermal stability (72, 73). In general, introduction of one additional double bond into a fatty acid at least doubles the rate of oxidation of the fatty acid or its esters (54). However, since conventional hydrogenation processes leads to formation of *trans* fatty acids, use of hydrogenated fats in frying mixes is no longer acceptable. In response, the food industry is moving to replace the saturated fatty acids with unsaturated fatty acids, such as oleic acid and linoleic acid which degrade rapidly during heating. New stabilization approaches are needed to successfully use these “healthy” oils in deep fat frying. The addition of the mono unsaturated oleic acid is an increasing trend, as it has only one unsaturation point and has been shown to have health benefits (60).

2.5.3 Controlling catalysts

Controlling catalysts in frying oil focuses primarily on improving oil refining and handling. The refining process removes phospholipids, free fatty acids, and most of the metals that can mediate secondary oxidations during heating and storage. Additionally, since metals are expected to be major catalysts throughout the lifetime of the oil, citric acid is added to commercial oils to chelate metals not removed by processing well as metals that make be picked up from processing equipment. Stainless steel 316 is recommended for use with oils since its surface is more impervious and it does not leach metals into the oil (23, 74).

Heat is difficult to limit since it provides the cooking during frying and each food has a different heat requirement. Nevertheless, it is prudent to use the lowest temperature consistent with complete cooking for each application (75). In addition, as noted above, continuous frying is better for the frying oil than intermittent cooking (40), and maintaining a constant temperature also reduces degradation.

Fryers are designed to limit catalytic factors as well. Cycling used oil through filters remove any food particles left that may be an additional source of metals (26).

2.5.4 Antioxidants

Use of antioxidants has been the most important approach to controlling the degradation of oil, other than limiting oxygen. Metal chelators that stop radical initiation are one important form of antioxidant. However, they must be supplemented with antioxidants that interfere with the propagation stage of lipid oxidation by quenching radicals and thus blocking radical transfer and formation of new radical chains (4, 50). Common chain-breaking antioxidants that have been used extensively in the food industry in the past include butylated hydroxytoluene (BHT) and *tert*-butyl hydroxyquinone (TBHQ) (7). Natural antioxidants that are found in oils, particularly mixed tocopherols, also retard oxygen's effect on the lipids (76).

Frying imposes considerable challenge in use of antioxidants. Antioxidants that are volatile (e.g. BHT) (61), oxidize readily (e.g. ascorbic acid), or induce charring (e.g. phosphoric acid and phosphates) cannot be used in oils intended

for frying (8). Radical quenching antioxidants that are very effective stabilizers at ambient or moderately elevated temperatures often seem to be much less effective in frying oils at temperatures >100°C. This loss of action at frying temperatures 175-200°C has most often been attributed to thermal degradation of the antioxidants themselves, or to antioxidant volatilization (29, 77). However, generally overlooked is an additional factor of extremely high radical loads generated by thermal scissions during frying. Reaction with these radicals, rather than direct thermal degradation, is the dominant mode of antioxidant depletion during frying and other thermal processing (50).

2.6. Methods of analyzing lipid oxidation in frying oils

Many different methods have been used to monitor lipid degradation in frying oils, including analysis of both physical changes and chemical products that occur during heating. For quality control in industrial laboratories where the focus is on oils already used, the most important physical changes are oil color, viscosity, and foaming (2). Volatile oxidation products such as hexanal are analyzed through the use of gas chromatography coupled with mass spectroscopy detection (78), while non- volatile oxidation products are analyzed by class in chemical assays as well as by high pressure liquid chromatography (HPLC) to separate and identify specific products. Key chemical assays for thermal degradation include peroxide values, polar products and total polar materials (which include carbonyls, carboxylic acids, and polymers as well as peroxides), and free fatty acids. Indeed, total polar products and free fatty acids

are used as the basis for legal limits on degradation to assure high quality and safety in frying operations. Legal limits in the United States are 27% total polar materials (unfiltered oil) or 24% polar products (filtered oil) and 2% free fatty acids (personal communication, M.M. Blumenthal, Libra Laboratories). Limits in Germany are 15% polars for industrial frying, 24% polars for commercial (restaurant) frying, 12% polymers (79).

These assays are useful tools for monitoring overall oil degradation during processing but are too crude for basic research where specific kinetics and mechanisms need to be determined. In research laboratories, monitoring oxygen consumption in an oxygen bomb can provide information about the kinetics of thermal degradation independently of specific products. This is an important adjunct to chemical assays since products and degradation pathways vary tremendously with frying conditions and foods.

Integrating oxygen consumption with multiple chemical and physical assays of lipid degradation that include both non- volatile and volatile products may be a time-consuming process, but it is necessary to develop the most accurate and complete picture of oil degradation, and it is critical for elucidation of degradation mechanisms.

2.6.1 Adaption of oxipres / oxygen consumption and oxygen bomb

An Oxipres™ oxygen bomb adapted for use at high temperatures (199°C) (Mikrolabs, Aarrhus, Denmark) has been demonstrated to be a very effective tool for following degradation of oils heated over a range of temperatures, headspace pressures and composition, and times (19, 20). In oxygen bombs, heating can be

controlled to 0.1 degree C and atmosphere can be precisely controlled to 0.01 bar; both can be varied to assess effects of temperature and oxygen. The Oxipres™ is a closed system that can be pressurized with any gas to pressures as high as 10 bars (5 bars is more typical). Below one bar pressure, the system can be used as a controlled reaction chamber but the pressure transducing sensor becomes too insensitive for accurate recording of oxygen consumption (19). High oxygen pressures prevent the system from becoming oxygen limited and provide a form of accelerated shelf life conditions at ambient to moderately elevated temperatures, but at frying temperatures can also obscure reaction pathways and shift product distributions unrealistically. Atmospheres of 2 bars air are more appropriate for frying studies (19).

Only small amounts (grams) of oil rather than gallons of oil need to be used, and both oil and headspace can be collected to analyze total non-volatile and volatile products as a function of heating time (19).

An important advantage of the Oxipres™ is that both oil and volatiles can be sampled at identical heating times and identified oxygen consumption levels. The collect total volatiles released by the oil during heating, the pressurized cells are vented through thermal desorption traps to collect and concentrate molecules in the headspace. Oil removed from the cell is analyzed for non-volatile products by any desired method, and volatiles collected on the trap are analyzed and identified by gas chromatography-mass spectrometry. Results of all products can then be integrated with oxygen consumption levels and rates to deduce reaction sequences active under various heating conditions.

The Oxipres™ has been used in Dr. Schaich's laboratory at Rutgers University to study lipid oxidation processes in frying oils as a function of oil and fatty acid composition, in margarine blends, animal fats, red versus yellow bone marrow, fish meals, and a wide range of dry food products (unpublished data).

2.6.2 Non-volatile chemical products (conjugated dienes, hydroperoxides, carbonyls)

Common non-volatile products used to follow lipid oxidation are conjugated dienes (CD), peroxide values (PV), and carbonyls.

Formation of conjugated dienes is the first change occurring in polyunsaturated fatty acids when hydrogens are abstracted to form the first radicals. The assay is simple and rapid and requires no reaction – oils or extracts are dissolved in cyclohexane or iso-octane and the absorbance at 234 nm is measured (80, 81).

Peroxide values (PV) measure formation and accumulation of lipid hydroperoxides (LOOH), the first stable oxidation product, as an indication for degradation. Peroxide assays are based on reactions of the –OOH group with various reagents, with quantitation by titration or by formation of a colored product that can be quantitated optically (82). Thus, PV assays present more inherent complications, but also have the disadvantage of recording only the amount of hydroperoxide present at the moment and this introduces considerable uncertainty in interpreting low values. Because hydroperoxides are decomposed by metals, light and heat, as discussed in previous sections, low PVs can indicate oxidation that is still in early stages or can reflect extensive oxidation that

has progressed beyond hydroperoxide decomposition. Hydroperoxide decomposition is particularly problematic at the elevated temperatures used in frying, and PVs thus must be accompanied by measures of secondary degradation products for accurate representation of the oil quality. Peroxide values are more appropriate for monitoring oxidation during shelf life studies at ambient temperatures (83), but are still used in frying research where high values indicate extensive oxidation. It must also be noted that current peroxide assays quantitate amounts present but do not distinguish the position of the –OOH group on an acyl chain or the fatty acid or fragment on which the –OOH resides.

Carbonyls compounds include aldehydes and ketones, and are secondary products resulting from lipid hydroperoxide degradation. The most infamous carbonyl is malondialdehyde, or MDA, which has been commonly used (and misused) as an indicator of lipid degradation through its reaction with thiobarbituric acid (TBA). However, MDA forms only in fatty acids with three or more double bonds and the TBA assay is not specific for malodialdehyde (84). Carbonyls are also measured by reaction with anisidine (85) or dinitrophenyllhydrazine (DNPH). Anisidine reacts only with 2-alkenals so does not detect all carbonyl products. Hydrazone products created by the reactions of the aldehydes and ketones with 2,4-dinitrophenylhydrazine (37) can be measured optically by their absorbance at 440 nm (24), or can be separated by HPLC to identify and quantitate specific carbonyl products (86). Carbonyl products form continuously throughout the frying process, indicating lipid degradation.

2.6.3 Volatile products

The importance of determining volatile products of lipid degradation is that volatiles not only are responsible for the characteristic flavors and odors associated with the fried food, but they are also the first indicators of rancidity for consumers and provide sensitive information that can be used to derive degradation pathways. Techniques most commonly used to analyze volatile thermal degradation products include static head space analysis, solid phase microextraction (SPME), and purge and trap (dynamic headspace analysis). The mode of collecting volatiles may be different in each technique, but the common factor is use of gas chromatography and mass spectrometry (GC-MS) as the method of choice for quantitation and identification.

2.6.3.1 Headspace analysis

Static headspace analyses sample volatiles that have been allowed to accumulate in a closed volume containing a test material. In static headspace analysis, an oil sample is placed in a closed vial, allowed to equilibrate at some elevated temperature, and the headspace vapors are withdrawn with a syringe and injected into a gas chromatograph for analysis. This technique is easy but lacks in sensitivity; it is used primarily with samples that have been stored (87).

2.6.3.2 Solid Phase Micro Extraction

Solid phase microextraction (SPME) uses a fiber coated resin onto which selected molecules will adsorb. The fiber is inserted through a septum into a vial containing a test sample and allowed to equilibrate with the headspace and adsorb volatiles, then transferred directly to the injection port of a GC, where it is

heated and the molecules are desorbed. SPME requires no solvents to facilitate volatilization, and offers particular advantage in concentrating adsorbed analytes, thus increasing sensitivity. Care must be exercised, however, in selecting resins with strong sorption of the analytes in the samples and in interpreting results. Results can easily be misinterpreted in terms of oxidation extent or pathways when the fiber has selective affinity for certain compounds while missing others (87).

2.6.3.3 Purge and Trap / Dynamic headspace

The purge and trap system (or dynamic headspace technique) allows for a more complete analysis of all volatiles and is more sensitive than static headspace analysis by a factor of 100 (88). P&T eliminates the need for any solvent extractions of the volatiles, reducing handling aberrations (89). In P&T sampling, inert gas is continually blown over the sample to collect volatiles then into a small column where volatiles are adsorbed onto a solid matrix and concentrated for analysis. This concentration is a critical component since it increases sensitivity and completeness of analysis – fewer volatile products are missed because of low volatility or dilution of headspace. Volatiles in the trap are then desorbed into a gas chromatograph for analysis.

In the current study, venting from the Oxipres™ was substituted for gas flow in loading the trap with volatiles. P&T provides a sensitive means of collecting volatiles in a closed system with greater recovery of all components.

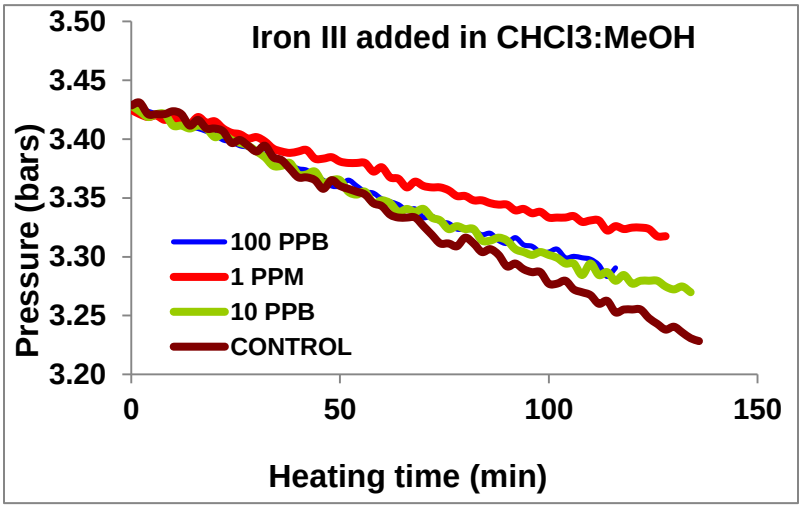
3. Hypothesis

Thermal scissions are the dominant reactions initiating and driving degradation of oils during heating, especially in early stages, and may obscure effects of normal pro-oxidants. Autoxidation becomes more important as secondary reactions with extended heating of oils.

3.1 Objectives

Objective 1. Collect volatiles from oils heated for up to three hours in an Oxipres™ operating at 180°C under 2 bars air. Determine whether volatiles released from heated oils contribute significantly to headspace of oxygen bomb and reduce apparent oxygen consumption.

This first objective evolved from testing the catalytic effects of various factors on oil degradation when heated in Oxipres™ oxygen bombs (20). Sometimes the oxygen consumption curves showed unexpected antioxidant effects. One example is shown in Figure 8, where there was a decrease rather than increase (catalysis) in apparent oxygen consumption when ferric iron was added to the oil to promote lipid oxidation. The sensor in the oxygen bomb is a sensitive pressure transducer rather than a specific oxygen sensor. This raises the possibility that volatiles released in active degradation could add to the headspace, increase the pressure, and counteract oxygen consumption. Objective 1, therefore, was to trap and quantitate total volatiles and determine whether this amount could feasibly account for aberrations in oxygen consumption curves (e.g. explain reduction of oxygen consumption by factors that are normally pro-oxidant.



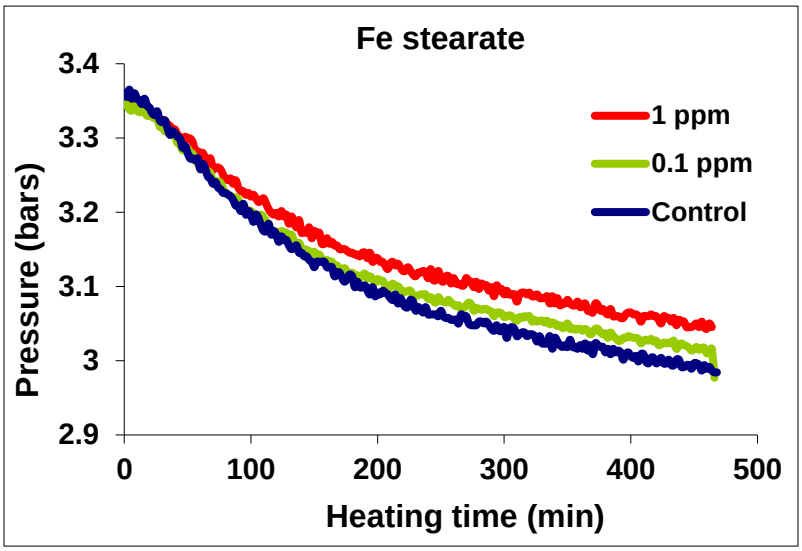


Figure 8. Effects of ferric iron (top) and ferric stearate (bottom) on oxygen consumption of a high oleic sunflower oil:corn oil 60:40 blend heated in an Oxipres™ oxygen bomb at 180°C for three hours under 2 bars air pressure. Data from (20).

Objective 2. Collect volatiles from oils heated for up to three hours in an Oxipres™ operating at 180°C under 2 bars air. Determine amounts and identities of volatile products to support chemical analyses of products and assess relative contributions of thermal scission from autoxidation reactions.

The second objective also evolved from the same oxygen bomb studies of thermal degradation of oils (20). Samples heated in oxygen bombs were withdrawn at 15 minute intervals and analyzed for lipid oxidation products (conjugated dienes, hydroperoxides, aldehydes, free fatty acids, polymers) to track what chemical changes were occurring as oxygen was consumed. In contrast to normal autoxidation, unusually high levels of aldehydes were observed (Figure 9 top), as well as an unusual product patterns (Figure 9 bottom). Hydroperoxides accumulated rapidly then decomposed, as would be expected under high heat conditions. Conjugated dienes, aldehydes, and polymers formed in parallel but after hydroperoxide decomposition. Fatty acid production began only after considerable delay. These were class analyses, detecting only specific functional groups, so they could not distinguish products arising from thermal scission from products generated by chain reactions of autoxidation. Objective 2, therefore, was to identify individual volatile products to corroborate chemical analyses, explain the unusually high aldehyde production, and shed light on the underlying chemical reactions.

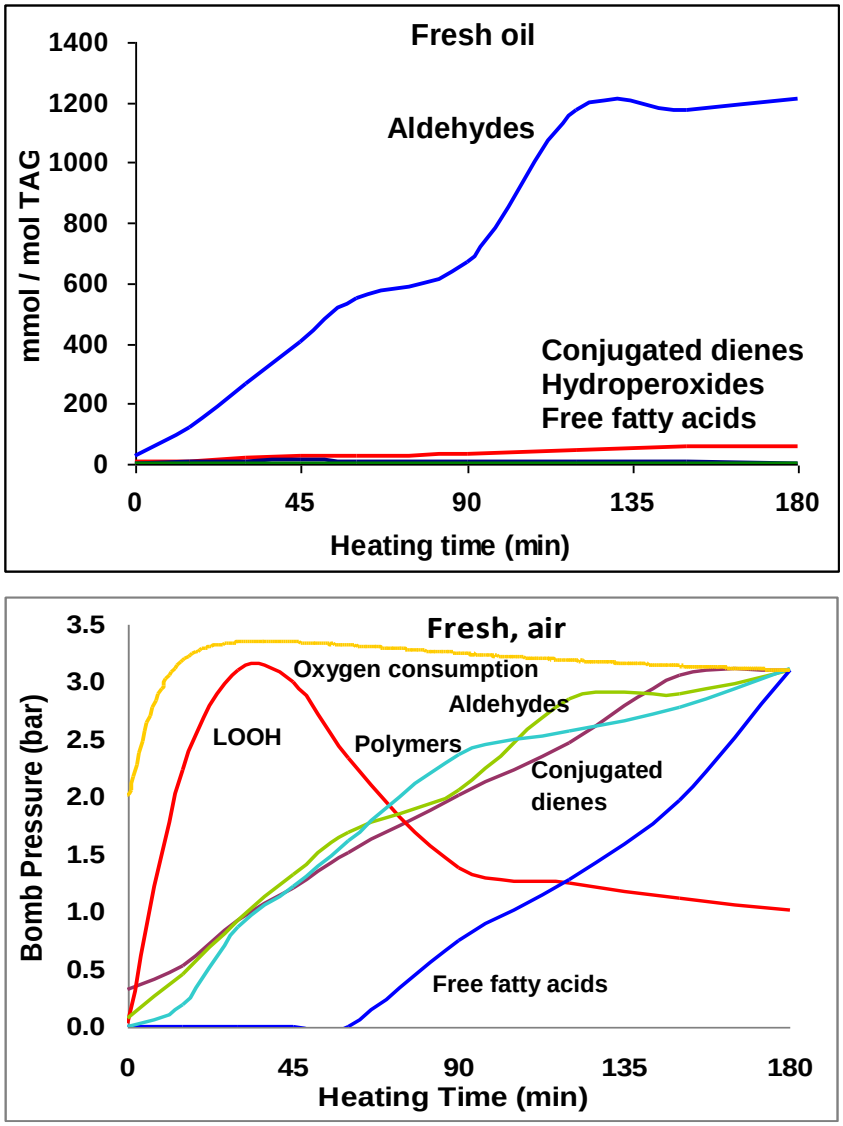


Figure 9. Generation of lipid oxidation products during heating of a freshly refined corn oil-high oleic acid sunflower oil blend at 180°C under 2 bars air for three hours. Data from (20). Top: Lipid oxidation product formation graphed on basis of absolute concentrations. Bottom: Lipid oxidation products normalized to oxygen consumption to show relative kinetics of formation of different products on the same graph.

4. Materials and Methods

4.1 Materials

The oil analyzed in this experiment was a 60:40 blend of high oleic sunflower oil (HOSO): corn oil mixture (gift of Frito-Lay North America, Plano, Texas). The fatty acid composition of the blend was 51% oleic acid, 38% linoleic acid, 7% palmitic acid, 3% stearic acid, and ~0.5% linolenic acid. Three different types of the 60:40 oil blends were used in this study: fresh oil, stripped, and steady-state oil. Fresh oil, protected during refining and storage, had very low levels of lipid degradation. It provided a model of oil in early “Fresh” stages of frying when few surface-active products are present (27). Stripped had metals, pigments, antioxidants, free fatty acids reduced to very low levels. Steady-state oil was preheated for several hours to generate surfactants and low levels of oxidation products comparable to those in steady-state oil used for industrial frying.

A series of alkanes and aldehydes, such as: 2- heptanone, nonanal, trans-2-heptenal, hexanal, octanoic acid, 2- nonanone, 2-pentanone, heptane, octane, nonane, decane, undecane, dodoecane, tridecane, heptanoic acid, 2-pentylfuran, octanal, trans-2-octanal, propionic acid, butanol, hexanoic acid, propyl acetate, hexyl formate, 1-octene, heptanal, and decanal. were used to verify elution order when comparing data from the HP gas chromatograph (used for quantitation) vs Varian GC-mass spectrometer (used for product identification). These were purchased from Sigma- Aldrich (St. Louis, MO). An

Equity-5 fused silica capillary 60 m x 0.32 mm x 1.0 um film thickness gas chromatography column was obtained from Supelco (Bellefonte, PA).

4.2 Methods

4.2.1 Overall experimental design

Samples of fresh and steady-state oils alone and with added pro-and anti-oxidant factors were heated for three hours at 180°C in an Oxipres™ oxygen bomb pressurized with 2 bars air. Oxygen consumption was monitored during heating, then non-volatile products were measured by chemical analyses and volatile products were determined by gas chromatography (Figure 10).

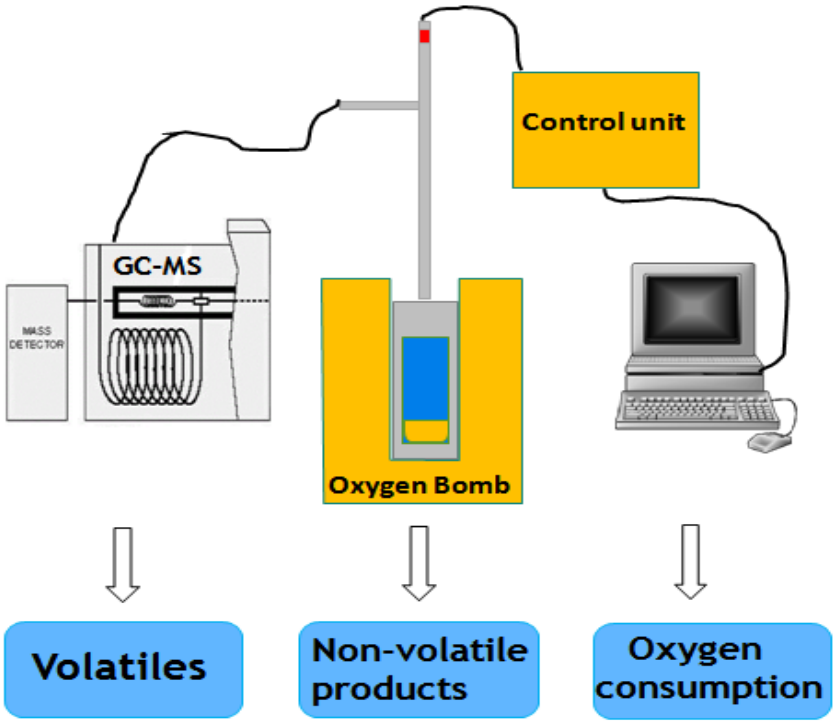


Figure 10. Flow diagram of overall experimental design for coordinated study of oxygen consumption, soluble oxidation products, and volatile products from oils heated at 180°C with various pro- and anti-oxidant factors added.

4.2.2 Oxipres™ oxygen bomb

Oil samples were heated under controlled temperature and pressure in an Oxipres™ Oxygen Bomb (Mikrolab, Aarhus, Denmark). For each test, ten grams of 60:40 high oleic sunflower: corn oil blend were weighed into sample flasks, then the flasks were transferred to the oxygen bomb cells, sealed and pressurized with two bars air, and placed into the heating unit (Figure 11). The chamber was heated to 180°C for up to three hours, and oxygen pressure was recorded using the Oxipres™ software. At the end of the heating period, the cells were opened and volatiles from the headspace were venting through and trapped on thermal desorption traps (Figure 11).

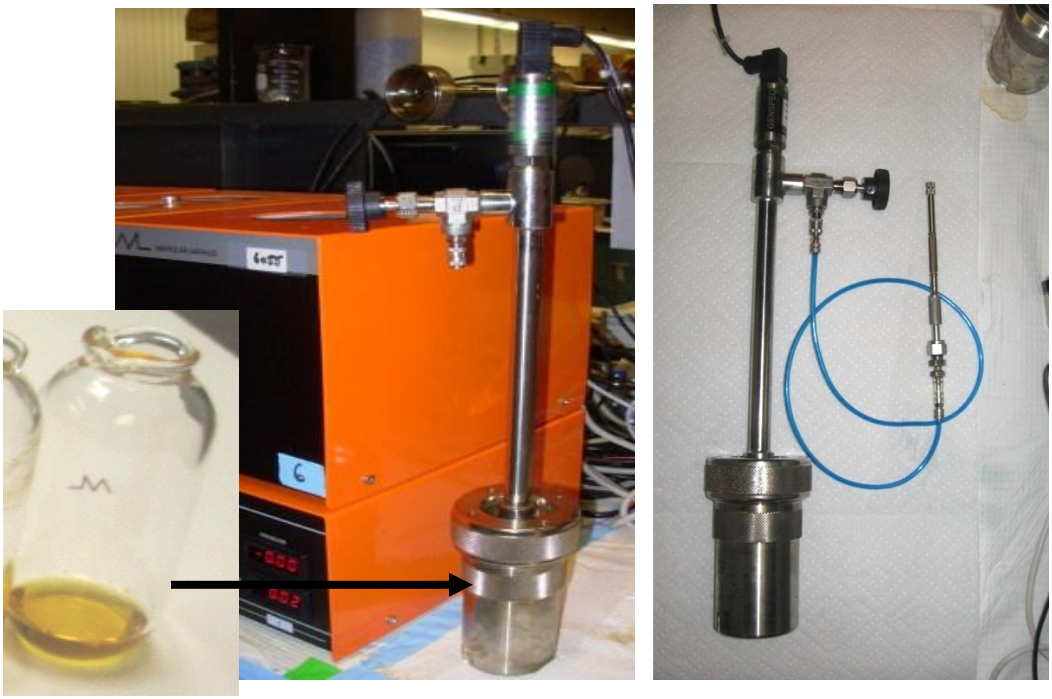


Figure 11. Oxipres™ oxygen bomb instrumentation. Left: Heating block, sample vial, and pressurized sample cell. Right: Connection of sample cell to Tenax trap for collecting vented volatiles upon release of pressure on cell.

4.2.3 Thermal Desorption Traps

Two types of thermal desorption traps were used to collect volatiles. Tenax-Carboxen 569 (1:1, 50 mg each) traps were used for dry samples In general; the Tenax packing adsorbs the larger molecules while the Carboxen 569 adsorbs the smaller ones. Samples that contained some water were trapped with100% Tenax, which has a lower affinity for water adsorption than Carboxen 596. To capture the volatiles, the trap was attached to the vent of the Oxipres™ via a piece of thin tubing while the headspace was vented from the Oxipres' chamber. The trap was then placed onto a TD-1 thermal desorption unit that was attached to a 5890 series Agilent gas chromatography. A 50mm injection needle was attached to the trap and used to inject the volatiles into the inlet of the GC.

4.2.4 Short Path Thermal Desorption

A Model TD-1 thermal desorption unit (Scientific Instrument Services, Ringoes, NJ) was attached to the inlet of a Hewlett Packard 5890 gas chromatograph. The heater blocks of the TD-1 were used to heat the thermal desorption trap to 250°C. The pressure of the TD-1 unit and the headspace of the GC were both set for 20 psi. The trap and needle were flushed with helium for 10 seconds before sample injection into the inlet. The injection was for 30 seconds so that the pressure between the GC and TD-1 could equalize. Flushing and injection times were monitored with a stop watch. The heating blocks of the TD-1 that encased the trap continued heating for five minutes so that the volatile thermal degradation compounds could be desorbed from the packing material of

the trap. Helium was also used as a carrier gas through the TD-1 thermal desorption unit and into the GC during the five minutes of heating

4.2.5 Gas Chromatography

To analyze the volatile compounds, a Hewett-Packard 5890A series 1 (Agilent, Santa Clara, CA) was used with a 100:1 split vent. The inlet was set for 250°C and had a straight liner. The oven temperature ramp was set at –20°C or 5°C for five minutes and then 10°/ minute until 280°C was reached, then held for twenty minutes. Dry ice was used to reach the –20°C initial temperature programming for dry samples and 5°C for samples that contained water. A flame-ionizing detector (FID) was used for the peak detection and its temperature was programmed at 290°C. Helium was used as the carrier gas through an Equity-5 fused silica capillary 60 m x 0.32 mm x 1.0 um film thickness. The head space pressure was held constant at 20 psi. An AMD2000 flow meter was used to monitor the flow rate through the split vent of 3 ml/min. A Chemstation10.9 computer program collected the data from the FID, and integrated areas under the peaks.

Peaks were identified using a Varian GC with the above mentioned programming connected to a Finnigan MAT 8230 Mass Spectrometer equipped with a Finnigan MAT SS300 data system. The ionization mode was electron impact mode (EI) at 70 eV with a position ion charge. The ion source temperature was 250 °C with the filament emission current set at 0.5 mA. The mass range was set for 35-350 Da with a scan rate of 0.6 s/d and an interscan time of 0.8 seconds. The resolution was set for 1000.

Identification of the peaks was achieved by a combination of a Varian gas chromatography- Finnigan MAT 8230 mass spectrometry (GC-MS) and elution order. The elution order is the specific sequence of molecules from the gas chromatogram. The mas spectroscopy identifies each molecule based up each “fingerprint” that a molecule gives off, it can help identify specifically the different types of volatiles from lipid degradation. Since the Hewett-Packer Agilent 5890A was not hooked up to a mass spectrometer, the elution order was established by six oil blend samples, control and catalysts, that were run on the Varian gas chromatography- Finnigan MAT 8230 mass spectrometry (GC-MS). The MS data (the peaks) were identified using the NIST Library and retention indexes. This then established the elution order in which to identify the peaks from the chromatograms of the Hewett-Packard 5890A series 1 GC. To ensure that the elution order was correct, standards were selected to verify some of the elution order. This included a series of alkanes and aldehydes, such as: 2- heptanone, nonanal, trans-2-heptenal, hexanal, octanoic acid, 2- nonanone, 2-pentanone, heptane, octane, nonane, decane, undecane, dodoecane, tridecane, heptanoic acid, 2- pentylfuran, octanal, trans-2-octanal, propionic acid, butanol, hexanoic acid, propyl acetate, hexyl formate, 1-octene, heptanal, and decanal. These standards were used to help verify some of the peaks on the Hewett-Packard 5890A. As an extra measure for correct peak identification, the standards were run with internal standards and the ratio between the internal standards and the standards was then calculated. Since the molecules always elute at the same

place, the ratio can be established and applying it to the chromatograms helped verify the correct identity of the eluted peak.

Peaks retention times and areas were calculated from runs on the HP 6890 gas chromatograph, using ChemStation software. The raw data was then transferred to Excel for determination of concentration by comparison to standards as described below, and for compilation.

4.2.6 Internal Standards

An internal standard mixture containing equal portions of benzene d-6, toluene d-8, and naphthalene d-8 at a 10mg/ml concentration in methanol, was spiked into the traps at 1µl using methanol to flush the needle. These were chosen as internal standards because they are not found in nature and therefore could be easily identified on the chromatogram. After spiking the traps, nitrogen was then run through the traps for 3 minutes to ensure adsorption onto the packing material. The internal standard peaks were used as guidelines in the elution order of the volatiles. Since the internal standard mixture was injected at a known concentration, it was also used to quantify the volatile concentrations in the trap by dividing the peak area of the internal standard by the peak areas of the thermal degradation volatiles, then multiplying by the internal standard concentration (10mg/ml). The concentrations of the volatiles in the trap were then reported in µg/trap.

4.2.7 Conversion of micrograms volatiles trapped to mmols trapped for comparison with oxygen consumption

Volatile contributions to headspace were calculated from the combined gas law equation PV=nRT, where R=8.314472 $cm^3 \cdot MPa/(K \cdot mol)$, T=453.15 K (180°C), $V_{headspace}$=162ml, $P_{measured}$ = Oxipres data converted to MPascals. Total volatiles were calculated summing all peaks the chromatograms (excluding internal standards), converting to µg/trap by comparison to the internal standards. This approach assumes that all volatiles were trapped when the Oxipres cells were released, and that the volatiles quantified in the GC chromatogram are a complete accurate representation of the total volatiles generated from lipid degradation. The total weight of volatiles thus obtained was then divided by the molecular weight of hexanal (100.16), a product mid-range in the molecular weights observed, to estimate the moles of volatiles released from each sample. Since the sum totals of the volatiles were recorded in µg/trap, the final number was divided by 1000 to convert µg to mmol.

5. Results and Discussion:

5.1 Gas Chromatograms

Gas chromatograms of volatiles presented fingerprints of degradation in the oils during heating. Figure 12 shows a typical chromatogram of fresh oil heated in air, obtained using the Hewett-Packer GC without MS detection. For all the data presented here, peak identities were determined from mass spectrometry detection on the Varian GC system and confirmed by authentic standards on the HP system.

The pattern of homologous series of compounds from C1 to C12 is certainly inconsistent with the selective, specific radical attack only at carbons allylic to double bonds and with subsequent hydrogen abstraction of lipid oxyl radicals (only traces of alcohols), but does support thermal scissions occurring at all positions of the acyl chains, as proposed by Nawar. Patterns of volatiles were similar throughout all of the different factor tests, with the majority of the peaks eluting before 30 minutes retention time. Approximately 72 peaks were detected using the GC-MS in each of the samples, but only about 42 were identified using the elution order due to coelution of some volatiles and different peak sensitivity between the Varian GC-MS and the Hewett- Packer 5890A GC with the thermal desorption unit. Co-elution of some compounds occurred because the temperature ramp of 10 °/minute that was mandatory for the GC-MS system was too fast to resolve compounds that were closely related in structure or overlapping in eluting power. Nevertheless, the MS software was able to identify

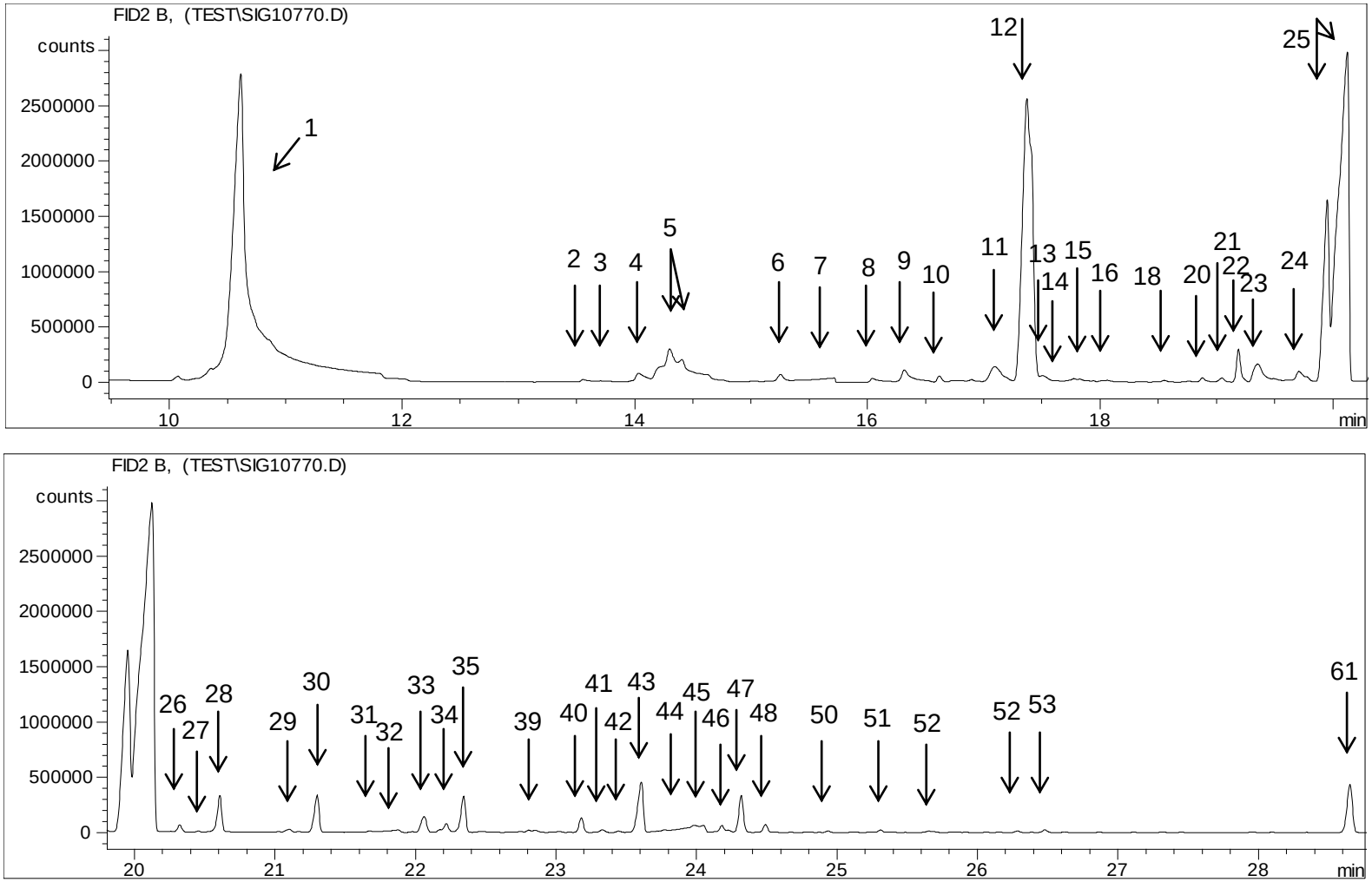


Figure 12. HP from Fresh oil blend heated at 180°C under 2 bars air for 180 minutes.

Figure 12 (legend). Compounds corresponding to labeled peaks.

1. acrolein + isopentane + n-pentane*
2. propanal
3. 2- butenal
4. n-propanol
5. hexene isomer + 2- butanone
6. methyl cyclopentane
7. formic acid
8. acetic acid*
9. d- 6 benzene internal standard
10. heptene isomer
11. 2- pentanone
12. pentanal* + n-heptane*
13. propyl acetate*
14. ?17.775
15. butyl formate
16. propionic acid*
17. ?18.371
18. 3-penten-2-one
19. ethyl cyclopentane
20. 2- pentenal
21. 3- hexanone
22. d- 8 toluene internal standard
23. pentyl alcohol
24. 2- hexanone/ 1-octene*
25. hexanal + n-octane + butyric acid
26. 2- octene
27. ?20.454
28. pentyl formate
29. 3- hexenal
30. 2- hexenal
31. ?21.675
32. pentanoic acid
33. 2- heptanone*
34. n-nonane*
35. heptanal*
36. pentyl acetate
37. ?22.75
38. ?22.807
39. hexyl formate*
40. butyl cyclopentane
41. cis-2-heptenal
42. ?23.446
43. trans-2 heptenal*
44. hexanoic acid*

Homologous Series

Alkanes and alkenes

Pentane	isopentane
Me cyclopentane	Et cyclopentane
Butyl cyclopentane	
Hexane	Hexene
Heptene	Heptene
Octane	1-Octene
Nonane	2-Octene
Undecane	
Dodecane	1-Dodecene

Acids	**Esters**
Formic acid	
Acetic acid	
Propionic acid	Propyl acetate
Butyric acid	Butyl formate
Pentanoic acid	Pentyl formate
	Pentyl acetate
Hexanoic acid	Hexyl formate
Heptanoic acid	Heptyl formate
Octanoic acid	Octyl formate
Nonanoic acid	

Aldehydes

Propanal	Heptanal
2-Butenal	cis-2-Heptenal
Pentanal	trans-2-Heptenal
2-Pentenal	Octanal
Hexanal	Trans-2-Octenal
2-Hexenal	Nonanal
3-Hexenal	

Alcohols

Propanol
Pentyl alcohol
Heptenol

Ketones

2-Butanone	2-Heptanone
2-Pentanone	3-Octanone
3-Penten-2-one	
3-Hexanone	
2-Hexanone	
Cyclodecanone	

45. ?24.046
46. 3-octanone?
47. 2-pentylfuran *
48. octanal*
49. heptyl formate?
50. heptenol?
51. trans-2 octenal*
52. heptanoic acid*
53. n-undecane*
54. nonanal*
55. ?26.624
56. octyl formate?
57. octanoic acid*?
58. 1-dodecene
59. n-dodecane*
60. ?28.342
61. d- 8 napthalene internal standard
62. nonanoic acid?
63. cyclodecanone?
64. ?38.213
65. ?38.53
66. ?39.076
67. ?39.406
68. ?40.047
69. ?41.183
70. ?41.794
71. ?42.323

*indicates peaks that have been verified with standards

overlapping peaks because it records full mass spectra for each data point.

The three main peaks observed consistently contained co-eluting components: 1) retention time about 11 minutes, contained acrolein, isopentane and n-pentane; 2) retention time ~17 minutes, pentanal and n-heptane together; 3) retention time ~ 20 minutes, hexanal, n-octane, and butyric acid. Minor or secondary peaks (≥ ~1µg/trap) showed homologous series of short chain alkanes, alkenes, aldehydes, alcohols, ketones, and acids in most samples, but the concentrations varied with factors. Effects of different factors on thermal degradation products were assessed by comparing concentrations per trap for these three main peaks plus concentrations of lesser products in various classes (aldehydes, acids, ketones, and miscellaneous).

Notably absent in all chromatograms was 2,4 decadienal, a major scission product of linoleate C9 alkoxyl radicals that is known to increase at high temperatures (90). Lack of 2,4-decadienal was also observed in thermal desorption studies of corn oil degradation at various temperatures (51). Experiments are planned to determine whether corresponding C9 attack did not occur in these systems or the 2,4-decadienal was rapidly oxidized to hexanal or decomposed to 2,3- or 4,5 epoxy derivatives, thence to 2-octenal and acetaldehyde or to 2-octene and glyoxal (37, 91).

5.2 Objective 1: Do volatiles released from heated oils contribute significantly to headspace of the oxygen bomb and reduce apparent oxygen consumption.

Reduction of the apparent oxygen consumption (antioxidant effect) was observed for several factors expected to be pro-oxidants. One notable example was iron, added as stearate or chloride. One possible explanation of this phenomenon was that volatiles released from the oil added to the headspace, negating the effects of oxygen loss on the pressure transducer response. To evaluate this possibility, total headspace volatiles for each sample were determined, converted to mmols products, and then compared with corresponding oxygen consumption curves.

Using this method, pressure contributions from volatiles calculated for systems where catalysis was expected but reduced oxygen consumption was observed were actually quite small (Table 4). Thus, assuming that major losses of headspace did not occur during venting (no leaking was obvious), volatiles released from the degrading oil contributed too few molecules into the headspace to account for the observed reduction in oxygen consumption. Hence, the oxygen bomb curves accurately reflect the oxygen consumption of the samples, and the apparent antioxidant effects of iron and other factors are real. Note added in proof: Internal data of Mikrolab demonstrated that at lower temperatures, volatile emissions form samples were miniscule compared to the oxygen in the headspace (Vagn Hansen, Mikrolab, personal communication).

The analyses presented here are the first demonstration that the pattern holds even at high temperatures where degradation can be quite rapid and extensive.

Recognizing that the oils are heated under pressure raises an explanation for the low volatiles concentrations. Volatilization of molecules is a process that occurs in equilibrium with the headspace. Pulling a vacuum releases atmospheric pressure on a solution and increases volatilization of molecules. In contrast, pressurizing the system, as occurs in the Oxipres™ cells, represses volatilization, hence the low concentrations of volatiles detected. This phenomenon is probably why the Oxipres™ can function with a pressure transducer rather than an oxygen-specific sensor. Under the 5 bar normal operating conditions of the Oxipres™, counterpressure on the sample surface is so high that only traces of volatiles, at most, escape to interfere with measurements in the headspace.

Table 4. Mols of volatiles expected if observed reduction in oxygen consumption by various factors was due to released volatiles versus actual mols headspace volatiles in thermal desorption traps when cells were vented.

Sample	Conc.	Headspace volatiles (mmol) Expected[1]	Observed[2]
$FeCl_2$	100 ppb	0.25	0.0145
	10 ppm	0.28	0.0150
	1 ppm	0.46	0.0148
$CuCl_2$	100 ppb	0.40	0.0228
	1 ppm	0.62	0.0217
	10 ppm	0.57	0.0198
$CoCl_2$	100 ppb	0.35	0.0197
	1 ppm	0.47	0.0200
	10 ppm	0.56	0.0207
Oleic Acid	0.05%	0.27	0.0143
	0.25%	0.18	0.0197
	0.50%	0.23	0.0158
Stearic Acid	0.05%	0.21	0.0130
	0.25%	0.16	0.0138
	0.50%	0.14	0.0148
di-St-PC	0.02%	0.11	0.0061
	0.05%	0.11	0.0105
Oleic acid and $FeCl_2$	100 ppb	0.25	0.0116
	1ppm	0.28	0.0097
	10 ppm	0.46	0.0114

[1] mols (n) corresponding to unexpected reduction in oxygen consumption by various catalytic factors
[2] mols (n) volatiles actually detected in headspace

5.3 Objective 2a. Do amounts and identities of volatiles products support chemical analyses of products?

5.3.1 Quantitative and kinetic correlation of total volatiles with oxygen consumption and production of non-volatile products

A corollary of Objective 1 is, if the mols of volatiles detected do not correlate with expected shortfalls in oxygen consumption, do volatile levels at least correlate with oxygen consumption in general and with non-volatile oxidation products? Table 5 shows the relationship between oxygen consumption, levels of non-volatile conjugated dienes, hydroperoxides, aldehydes, and free fatty acids, and total volatile products trapped. Total volatiles change in the same manner as other products, demonstrating that volatiles are being produced from common processes. A critical difference, however, is that higher concentrations of volatiles are released than moles of oxygen absorbed. This is an important clue to mechanisms since, looking further into components of the volatile fraction, we can see that alkanes account for ~75% of the volatiles from fresh oils, and this level drops to about 55% in steady state oils (Table 6). Catalytic factors drop alkanes further to 30-38%. At the same time, oxygenated products, particularly ketones, increase in both of the latter situations.

That the alkanes are predominantly short-chain species and appear in a homologous series supports thermal scissions as the source of the alkanes, particularly in early stages of frying in fresh oils. However, alkane thermal scission products should have still been present in sparged N_2/Ar but are not. More detailed product analysis of the oil will be required to explain this behavior.

Table 5. Comparison of oxygen consumption, non-volatile oxidation products, and total volatiles in corn oil/HOSO blends heated at 180°C under 2 bars air pressure for 3 hours.

	Fresh	St State	N_2/Ar
Oxygen consumption[a]	78	104	nd
Conjug dienes[a]	53	70	16
PV[a]	2.7	3.0	2.7
Aldehydes[a]	135	85	nd
FFA[b]	0.99	1.22	0.17
Total volatiles[a]	106	153	4

[a] mmols product / mol triacylglycerols
[b] % free fatty acids, w/w

A dominant role for thermal scissions in initiating early oil degradation is also supported by the relative kinetic patterns of volatile and non-volatile product formation shown in Figures 13-17. In oil with no added factors, production of volatiles increased linearly ($y = 0.3046x - 3.0347$, $R^2 = 0.9992$) up to 120 minutes heating, then became more variable. Conjugated dienes initially lagged behind volatiles, reached the same production rate between 80 and 120 minutes, then declined in parallel with volatiles (Figure 14).

In contrast to the slow production of conjugated dienes, hydroperoxides increases rapidly from the start of heating, reached a peak at 50 minutes, and declined thereafter due to either reaction or thermal decomposition (Figure 15). Non-volatile aldehydes increased rapidly with LOOH, but volatile aldehydes showed a short lag phase until about 40 minutes and did not begin to accumulate until hydroperoxides began to decompose (Figure 16). This suggests that there

may be two mechanisms of aldehyde generation, one associated with initial scission processes and the other deriving from hydroperoxide decomposition to alkoxyl radicals which then undergo scission to aldehydes. These reactions will be discussed in more detail in later sections.

Both volatile and non-volatile fatty acids begin to accumulate shortly after 180°C is reached when the oils are heated in air (Figure17). The acids detected were all short chain acids from formic to octanoic. Obviously, these are detected in preference to long chain hydrolysis products due to volatility limits. Nevertheless, while the medium chain acids may appear as secondary scission products in autoxidation of free fatty acids, they are not found in autoxidation of oils (see Table 3), and the short chain fatty acids are not autoxidation products. The rapid appearance of free acids without water indicates that there must be a second mechanism for their production, other than hydrolysis. These issues will be discussed in more detail in the next section.

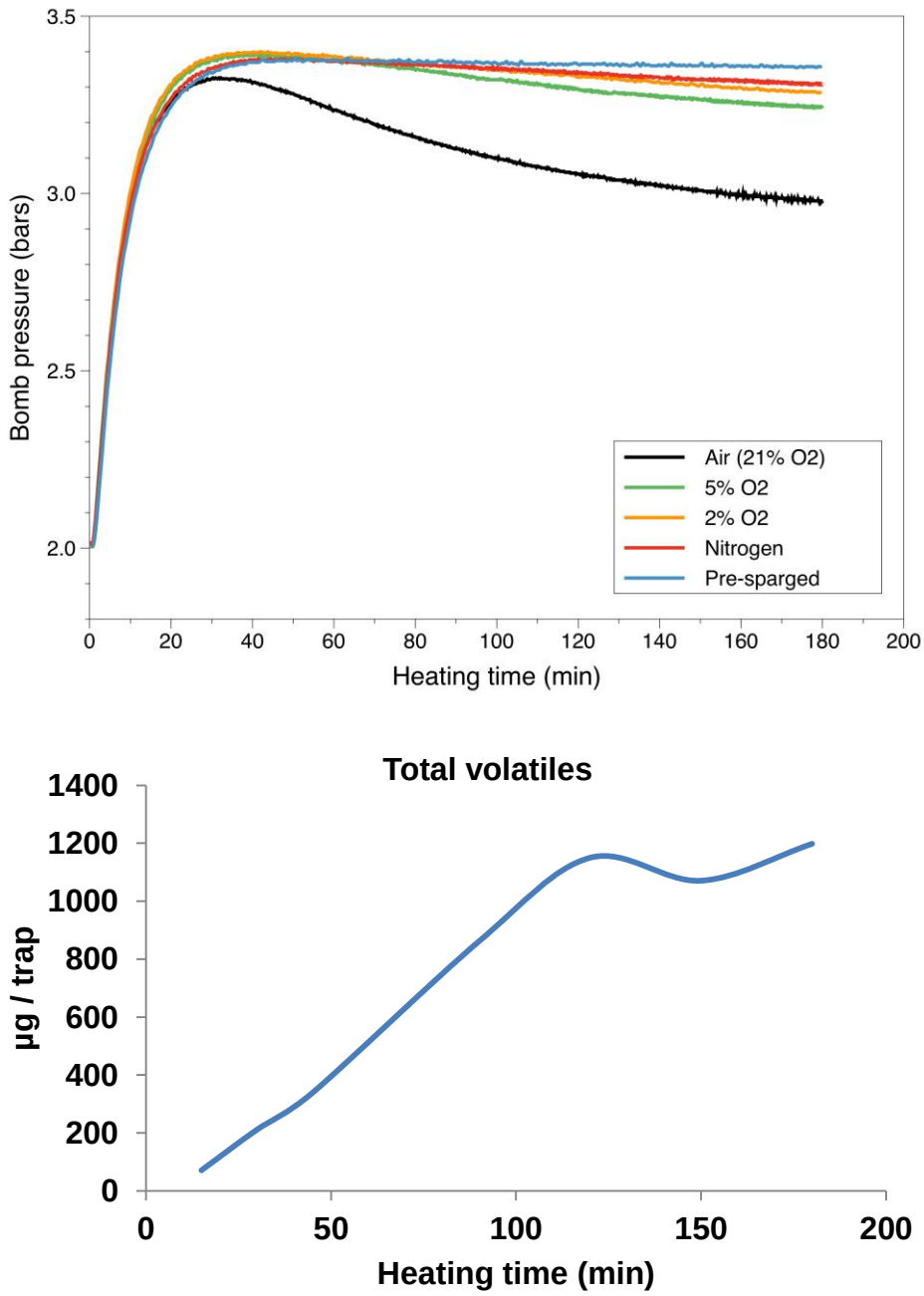


Figure 13. Comparison of oxygen consumption (top) and evolution of headspace volatiles (bottom) in fresh corn oil-HOSO blends heated at 180°C, for 180 minutes under 2 bars air. Oxygen consumption data from (20).

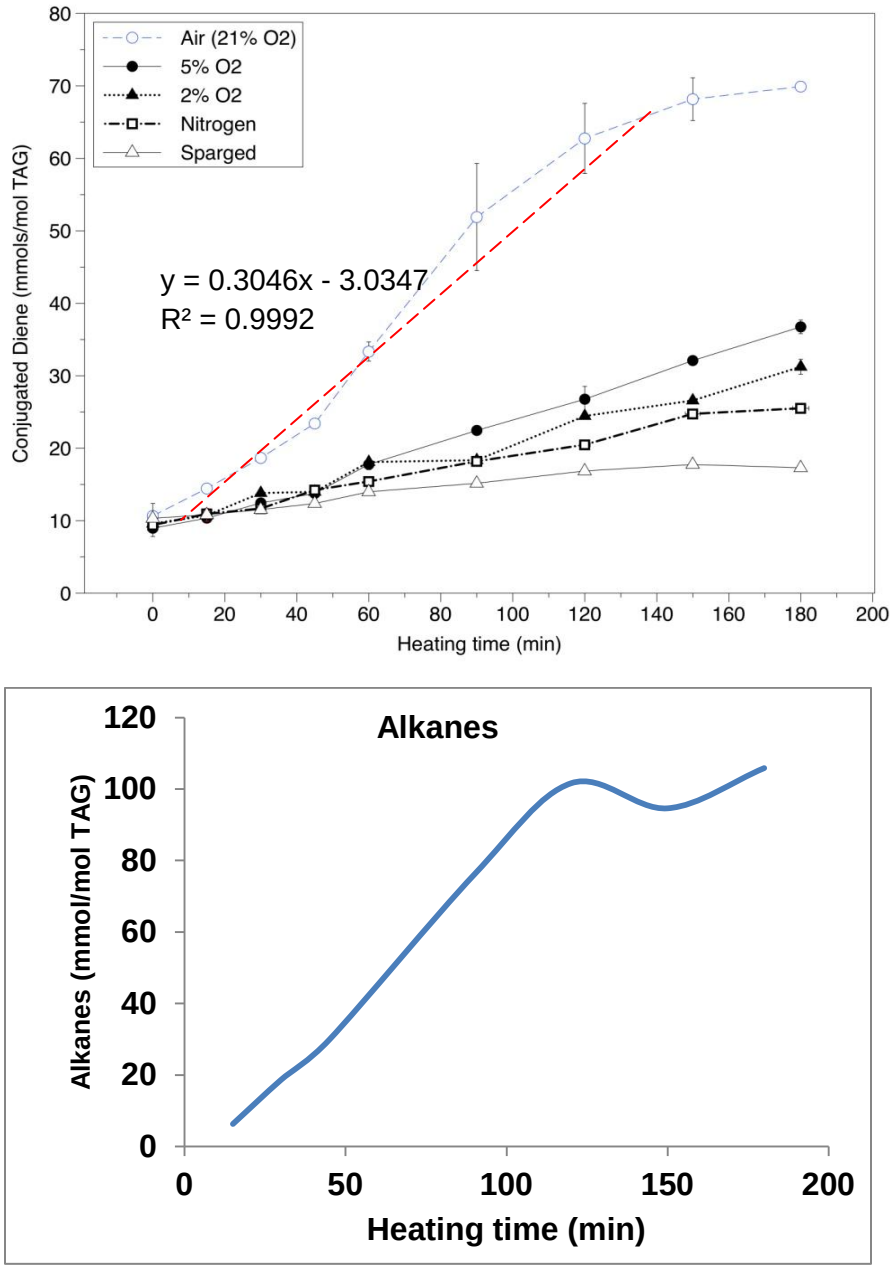


Figure 14. Comparison of conjugated dienes (H abstraction products, top) and evolution of headspace alkanes (thermal scission products, bottom) in fresh corn oil-HOSO blends heated at 180°C, for 180 minutes under 2 bars air. Conjugated dienes data from (20).

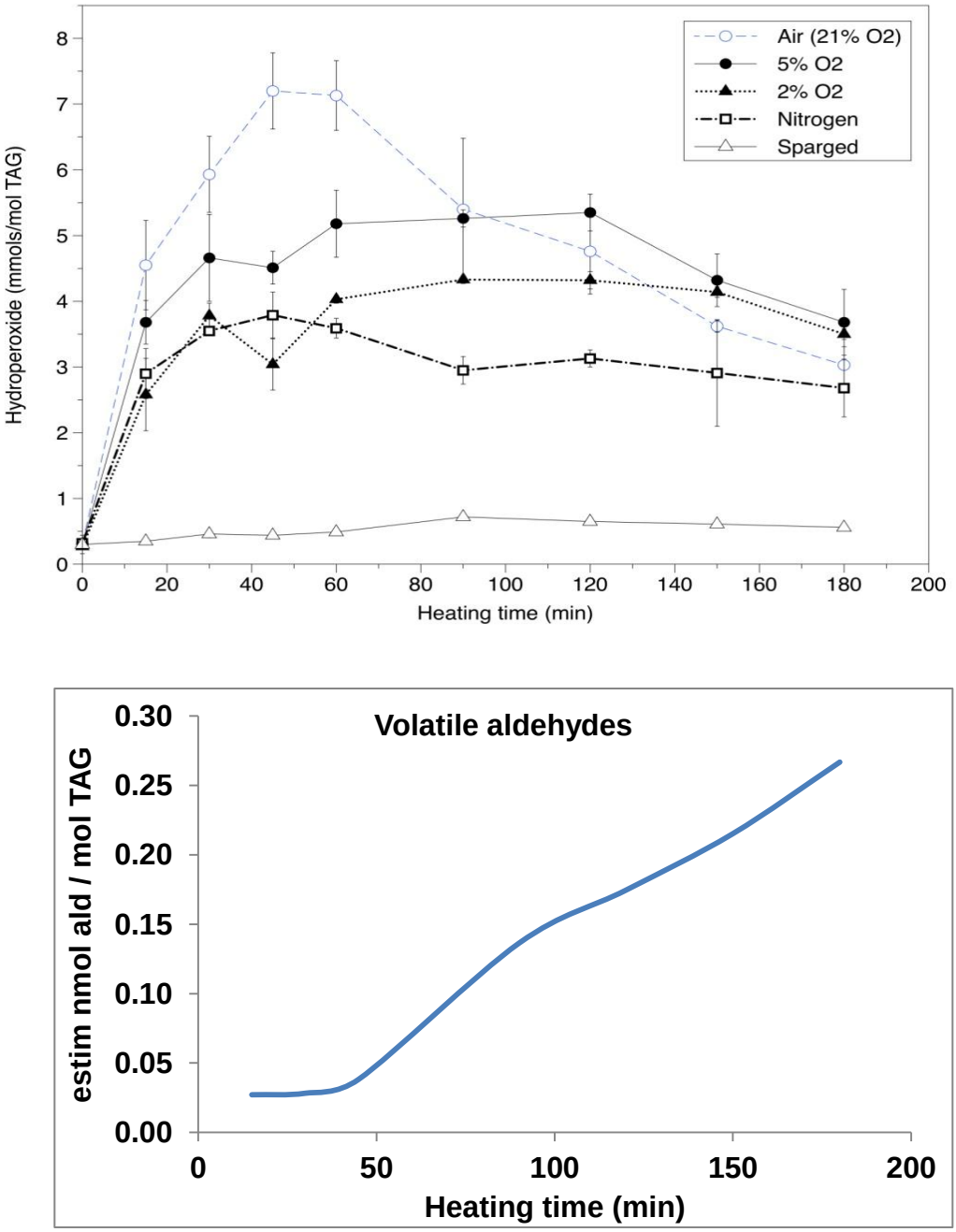


Figure 15. Comparison of formation kinetics for non-volatile hydroperoxides (top) and volatile aldehydes (bottom) in fresh corn oil-HOSO blends heated at 180°C, for 180 minutes under 2 bars air. Hydroperoxides data from (20). Molarity of volatiles estimated from mol wt of hexanal as average aldehyde size detected.

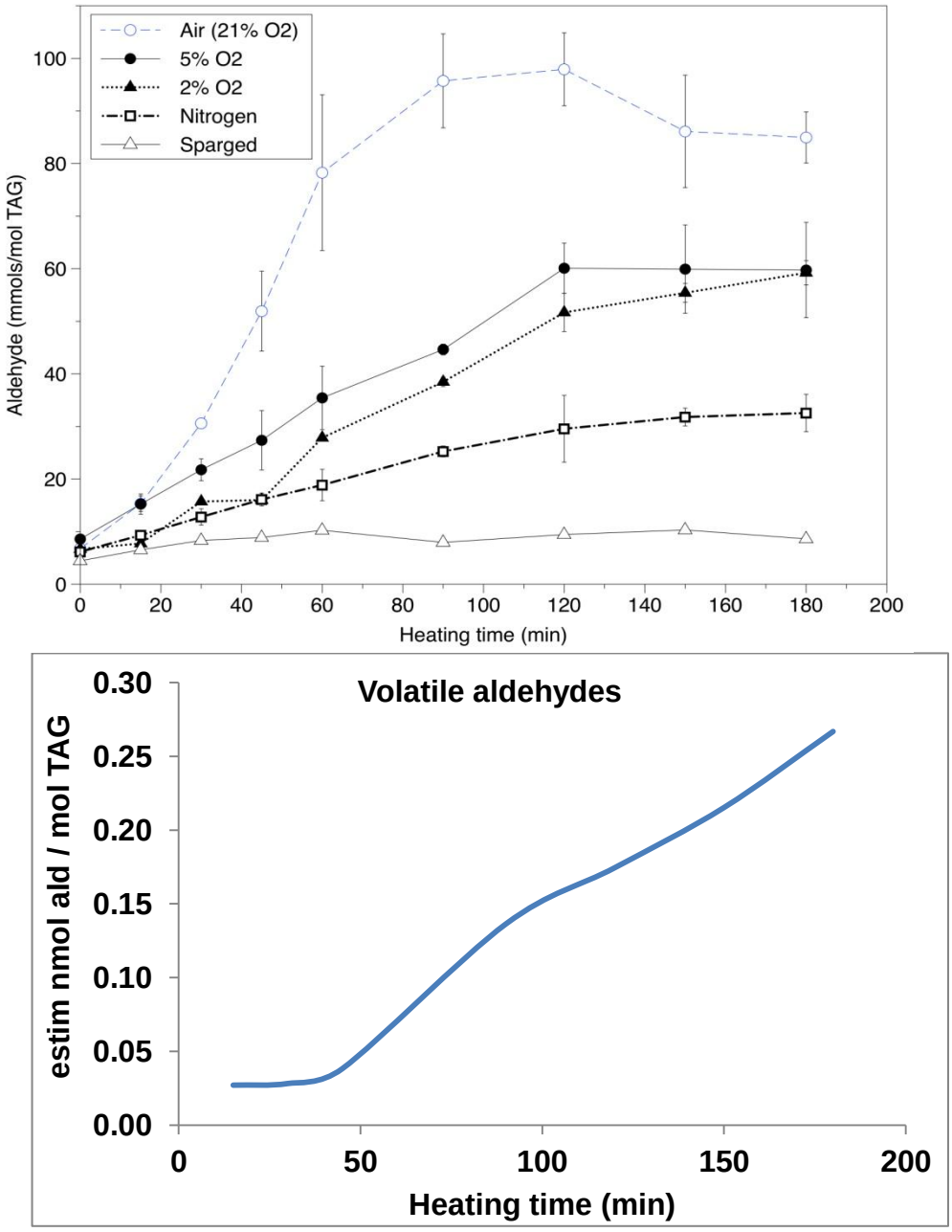


Figure 16. Comparison of formation kinetics for non-volatile (top) and volatile (bottom) aldehydes in fresh corn oil-HOSO blends heated at 180°C, for 180 minutes under 2 bars air. Non-volatile data from (20). Molarity of volatiles estimated from mol wt of hexanal as average aldehyde size detected.

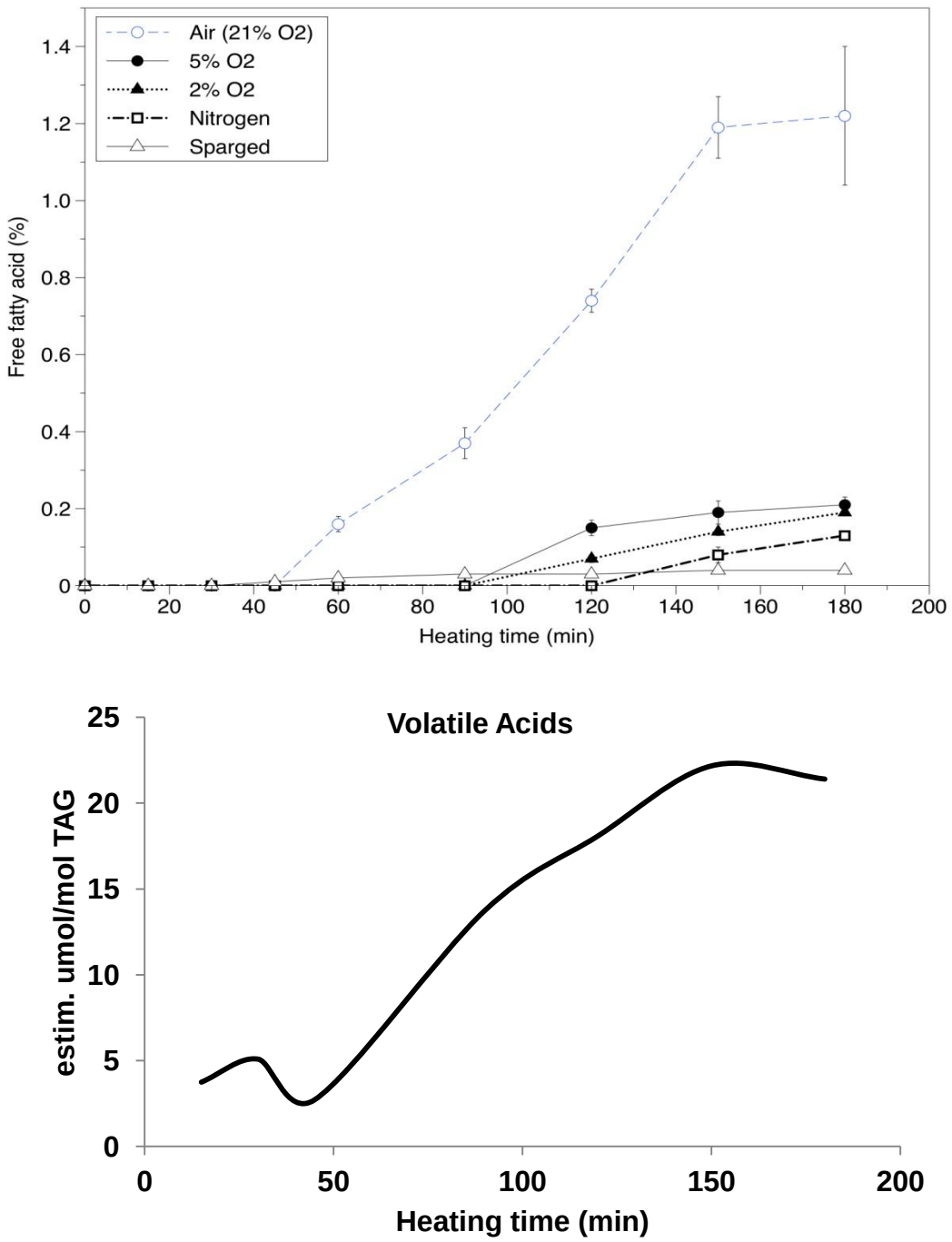


Figure 17. Comparison of formation kinetics for non-volatile free fatty acids (top) and volatile carboxylic acids (bottom) in fresh corn oil-HOSO blends heated at 180°C, for 180 minutes under 2 bars air. Non-volatile data from (20). Volatile acid molarity estimated from butyric acid mid-range between C1 and C9.

5.4 Effects of catalytic factors

5.4.1 Air (oxygen) vs argon

To investigate the specific production of thermal scission products, oil was heated under air and under nitrogen after sparging the oil with argon to deplete dissolved oxygen. It was expected that exclusively alkanes would be produced under inert atmosphere and this would represent products from thermal energy deposited in the acyl chains, while products from the oxidation of thermal scission radicals plus autoxidation chains initiated by hydrogen abstraction would be added when the oil was heated in air.

As shown in Figure 18, alkanes were by far the dominant product under all conditions. Under nitrogen after argon sparging of the oil, alkanes increased rapidly, reached a maximum within 45 minutes, and then declined. In contrast, under air, alkane production was slower but continuous and did not reach a maximum until 120 minutes and thereafter declined. Although thermal scissions are a constant process as long as heat is applied, accumulation of scission fragments in the oil creates an unstable situation. At some point, high enough concentrations are reached that radical recombination becomes faster than radical scission, and dimerization or polymerization ensues. In sparged argon, there is no competition for the thermal energy or scission radicals so they accumulate rapidly and reach the critical recombination or bimolecular concentration sooner, then levels detectable as volatiles decrease as recombination converts monomers to dimers and perhaps polymers. In air, in contrast, oxygen adds to some of the fragments and diverts them to oxidation

products, so accumulation of alkanes is slower and reaches the critical recombination concentration after longer heating times.

Under air, the most important oxygenated product class was aldehydes. In air, there was a short lag time before aldehydes began to accumulate slowly but continually after alkanes (Figure 18) and hydroperoxides (Figure 16) had begun to form. On the other hand, in sparged argon/nitrogen, levels of aldehydes, ketones, and other oxygenated products remained approximately constant at low levels over the entire heating period. Low levels present probably resulted from traces of oxygen not removed from the oil.

Thus, thermal scissions appear to be the precursor for both hydroperoxides and aldehydes. The dominance of short chain aldehydes and very low levels or absence of medium chain aldehydes (C8-C12) suggest that normal oleic and linoleic acid hydroperoxides are not important intermediates, at least during short-term heating.

Of the other oxygenated products, acids and esters were probably present in low levels in the oil, and these decomposed during heating. That acids were not produced in sparged N_2/ Ar, ketones indicates that the volatile acids being detected in these systems were oxidation, not hydrolysis products. Under argon, furans and alcohols did not change over time. However, in air ketones, acids, esters, and aldehydes increased in parallel at about the same rate, suggesting a common precursor step. Furans and alcohols formed more slowly and at much lower levels, and clearly were mechanistically disconnected from the other products. Reactions explaining these patterns will be presented in a later section.

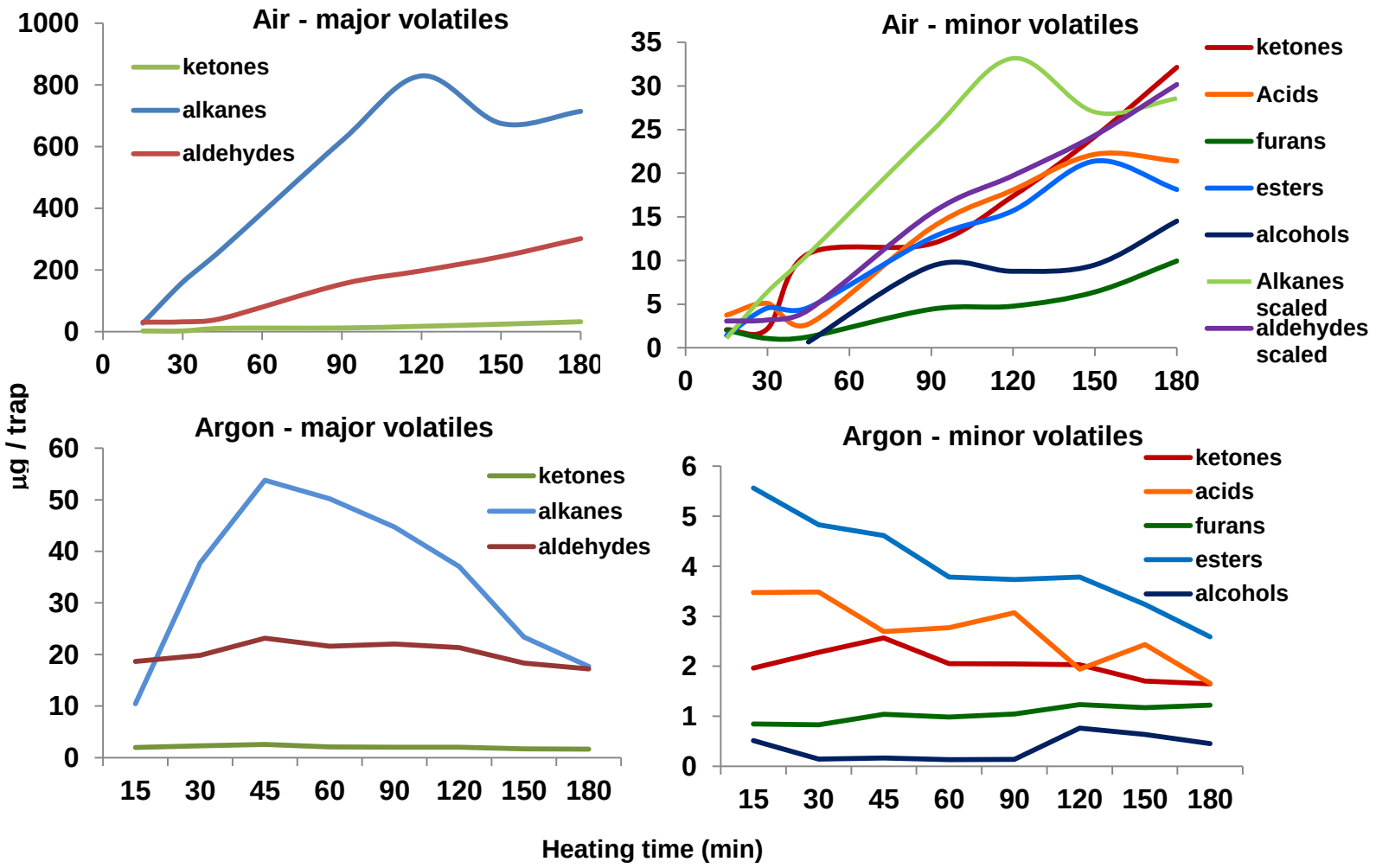


Figure 18. Effects of atmospheric oxygen on distribution of volatiles products formed in heated fresh oil corn oil-HOSO blends.

5.4.2 Effect of exposure surface area

Questions were raised about the limited oil surface area in the Oxipres™ cell compared to a much greater exposure in commercial fryers and very high surface to volume ratios in industrial-scale continuous fryers. To explore whether surface area could be a confounding factor, the oil was coated onto Celite or glass wool to spread it out over solid supports with two different chemistries.

Degradation increased on both solids supports, more on glass wool than on Celite (Figure 19). Alkanes nearly doubled on glass wool, but increased less than 25% on Celite. Aldehydes and other oxidation products increased on both supports. The one exception was alcohols, which decreased. Most changes were moderate, except for large increases in ketones (Figure 19) and unidentified products (data not shown) plus marked decreases in alcohols. These changes are consistent with metal contamination on the solid supports. In aprotic media, metals change reaction mechanism and catalyze oxygen insertions to form ketones and epoxides rather than decompose hydroperoxides or reduce oxygen (65). Thus, from product data alone, it cannot be ascertained whether increased degradation resulted from increased surface are and exposure on the solid supports or, rather, from metal contaminants on those supports.

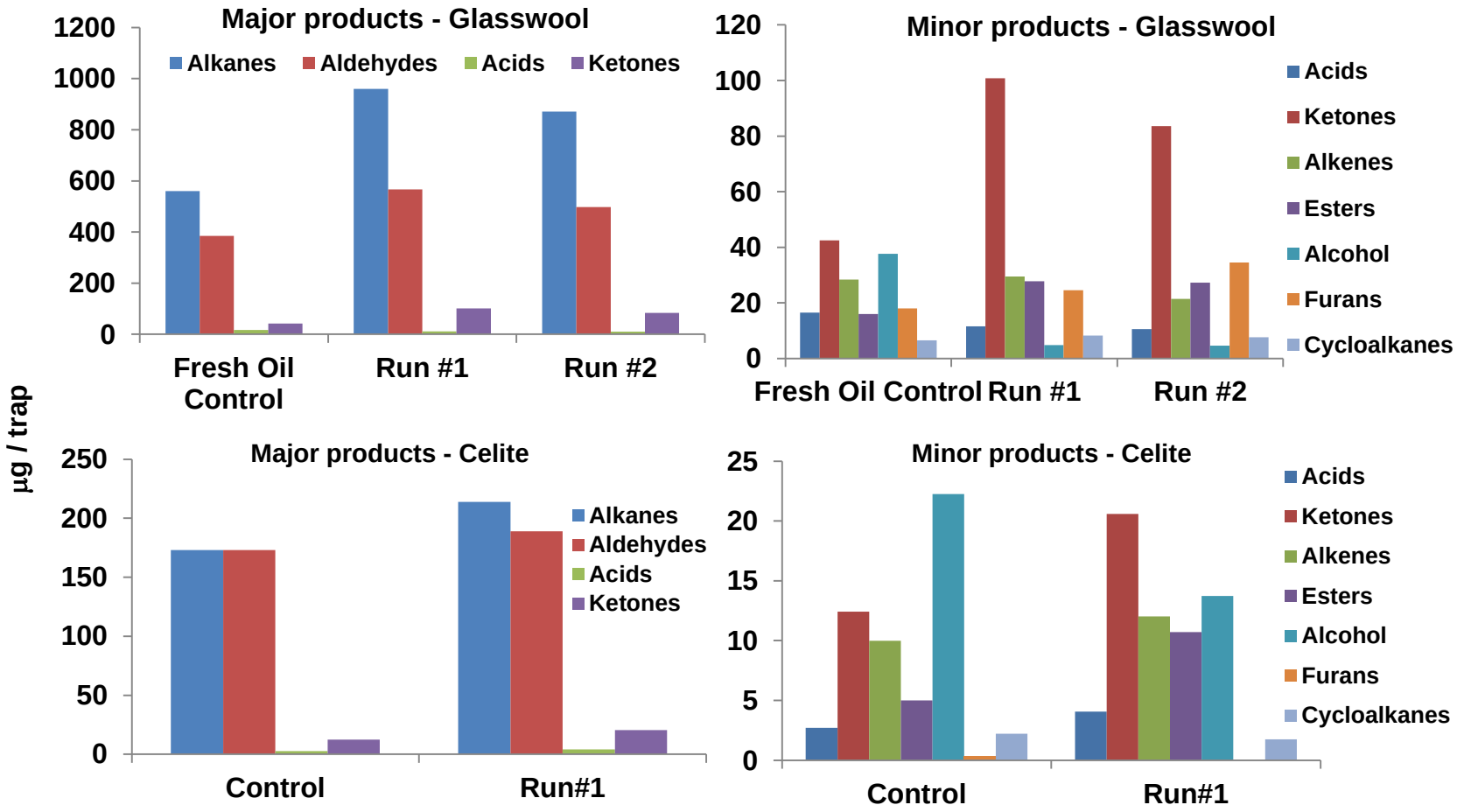


Figure 19. Effects of increased surface area on distribution of volatiles products formed in corn oil-HOSO blends heated at 180°C for 3 hours under 2 bars air pressure.

5.4.3 Effect of pre-oxidation: Fresh vs Steady state oil

Frying is never performed in fresh oil, but requires some pre-heating and oxidation to develop levels of polar surface-active products sufficient to mediate contact of the oil with the food (27). In addition, fresh oil has few molecular targets except the double bonds for catalysts, while the hydroperoxides, other oxygenated products, and acids in the steady-state oil used in industrial or commercial frying can become focal points for interactions with metals, fatty acids, and interactions that may both enhance primary degradation kinetics and dramatically alter the distribution of products. Thus, products in freshly refined and heated oil were compared with those in steady-state oil.

As expected, all classes of products except alcohols and unknowns increased in steady-state oil (Table 6). The ratio of products (steady state: fresh) did not vary much, indicating that the thermal processes still dominated over secondary oxidations and degradations. Increased ketones and acids result from secondary radical recombinations and oxidations.

Table 6. Volatile product distribution in fresh and steady-state corn oil/HOSO blends heated for 3 hours at 180°C under 2 bars air pressure.

	μg volatiles		
	Fresh oil	**Steady-state oil**	**Ratio St St/Fresh**
Alkanes	559.68	963.31	1.72
Alkenes	28.39	41.03	1.45
Aldehydes	385.05	621.20	1.61
Acids	16.52	34.06	2.06
Ketones	42.54	82.93	1.95
Esters	16.04	29.51	1.84
Alcohols	37.65	37.99	1.01
Furans	18.06	29.41	1.63
Cycloalkanes	6.53	10.20	1.56
Unknowns	8.34	6.10	0.73

5.4.4 Effects of catalytic factors: Metals, phospholipids, fatty acids, water

5.4.4.1 Metals

Effects of single catalytic factors on levels and distribution of volatile degradation products in corn oil/high oleic acid sunflower oil heated at 180°C for 180 minutes under 2 bars air pressure are summarized in Table 7 to provide an overview of all treatments. Effects of individual factors are illustrated in more detail in graphs in sub-sections following.

Volatiles generated in the presence of metals support the surprising antioxidant effects of iron observed in oxygen consumption analyses (20). Ferrous iron reduced the total volatiles and all products except carboxylic acids, but product distributions were not the same in steady-state versus stripped oils (Table 7). Concentrations of primary products were reduced more in stripped oils than in steady-state oils (Figure 20), but the reverse was true for secondary products (ketones, alkenes, esters, alcohols), which were higher in stripped oils (Figure 22). This difference may be due to citric acid that was present in the steady-state oil but not in stripped oil. $FeCl_2$ doubled carboxylic acid levels in steady-state oil relative to controls.

Effects of other metals are not so clear-cut. In steady-state oils, copper and cobalt reduced levels of oxygen consumption and non-volatile products (20), but increased total volatiles by 10-15%. $CuCl_2$ was most active at low concentrations (Figure 22), while cobalt was the reverse (Figure 23). Most importantly, both of these metals shifted the distributions of volatile products, where especially ketones were increased dramatically relative to the other

Table 7. Effects of single catalytic factors on distribution of volatile products in a steady-state corn oil/HOSO blend heated 3 h at 180°C.

	OC[1]	TV[2]	Alkanes	Aldehydes	Acids	Ketones	Alkenes	Esters	Alcohols	Furans	C-alkanes[3]	"X"
SS Control	106.54±0.02	1850	963	621	34	83	41	30	38	29	10	6
$FeCl_2$ Stripped												
100 ppb	79.52±0.52	1175	643	338	10	50	43	29	35	14	6	7
1ppm	78.78±10.98	884	511	226	11	36	33	20	28	11	4	4
10ppm	67.69±10.98	1202	717	307	15	53	32	40	22	6	7	3
$FeCl_2$ Steady-state												
100 ppb	79.52±0.52	1455	783	467	75	23	38	21	18	18	7	5
1ppm	78.78±10.98	1498	771	496	70	28	36	21	25	19	7	6
10ppm	67.69±10.98	1481	749	526	66	32	40	19	27	26	9	3
$CuCl_2$												
100ppb	73.60±2.62	2280	1208	742	52	133	44	24	25	29	11	11
1ppm	58.07±4.71	2178	1100	763	52	108	40	27	25	25	14	20
10ppm	61.77±0.52	1985	963	697	51	104	35	24	24	29	13	44
$CoCl_2$												
100ppb	82.11±3.14	1974	968	665	59	111	47	27	32	35	10	20
1ppm	73.60±4.71	2002	998	671	35	137	49	25	29	29	10	20
10ppm	67.32±6.28	2068	1030	702	58	129	44	26	30	30	8	11
di-O-PC Stripped												
0.02%	106.91±0.50	769	541	132	7	31	29	8	10	6	4	2
di-St-PC Stripped												
0.02%		609	428	103	3	11	16	16	9	4	3	6
0.05%		1055	506	368	6	24	34	21	30	27	5	16
di-St-PC Steady-state												
0.02%		1593	781	541	39	74	43	25	32	36	10	9
0.05%		1669	849	574	28	105	8	27	26	34	9	9
0.20%		1713	830	623	31	67	26	29	32	56	10	7

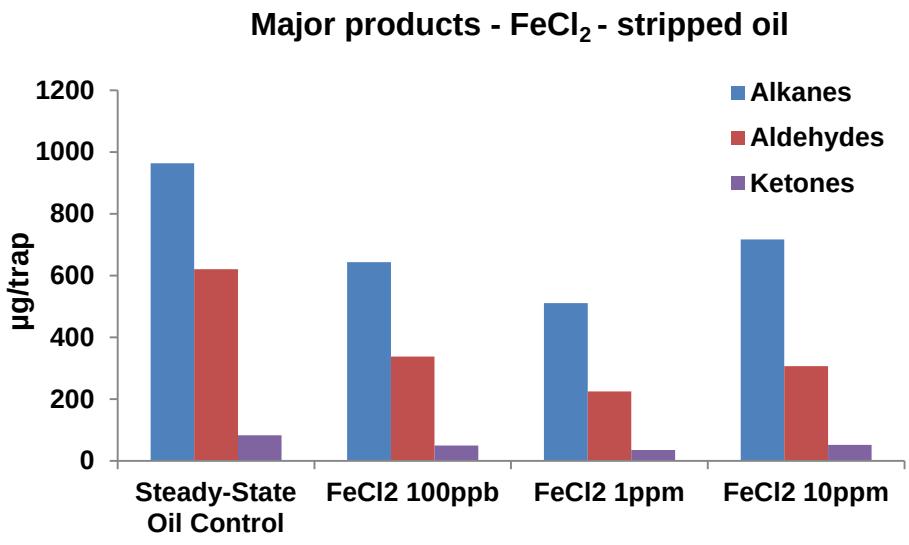
[1] OC= Oxygen Consumption, [2] TV= Total Volatiles, [3] C-alkanes= cycloalkanes

Table 7. Effects of single catalytic factors (continued).

	OC[1]	TV[2]	Alkanes	Aldehydes	Acids	Ketones	Alkenes	Esters	Alcohols	Furans	C-alkanes[3]	"X"
SS Control	106.54±0.02	1850	963	621	34	83	41	30	38	29	10	6
Stearic Acid Steady-state												
0.05% St	92.10±5.75	1300	643	445	32	72	51	19	22	22	8	8
0.25% St	95.06±12.03	1384	706	468	33	54	31	23	36	21	8	5
0.5% St	96.90±2.09	1480	755	511	25	51	26	31	35	28	9	8
Oleic Acid Steady-state												
0.05% O	87.29±6.28	1433	735	492	27	65	37	18	25	23	8	3
0.25% O	93.95±1.05	1973	1079	600	42	98	60	27	23	28	10	6
0.5% O	90.25±0.00	1584	848	511	34	51	63	22	14	24	9	9

[a] Units of products: Oxygen consumption in mmols O_2/ mol triacylglycerols
Volatile product concentrations in μg / headspace (from 10 g oil)

[1] OC= Oxygen Consumption, [2] TV= Total Volatiles, [3] C-alkanes= cycloalkanes



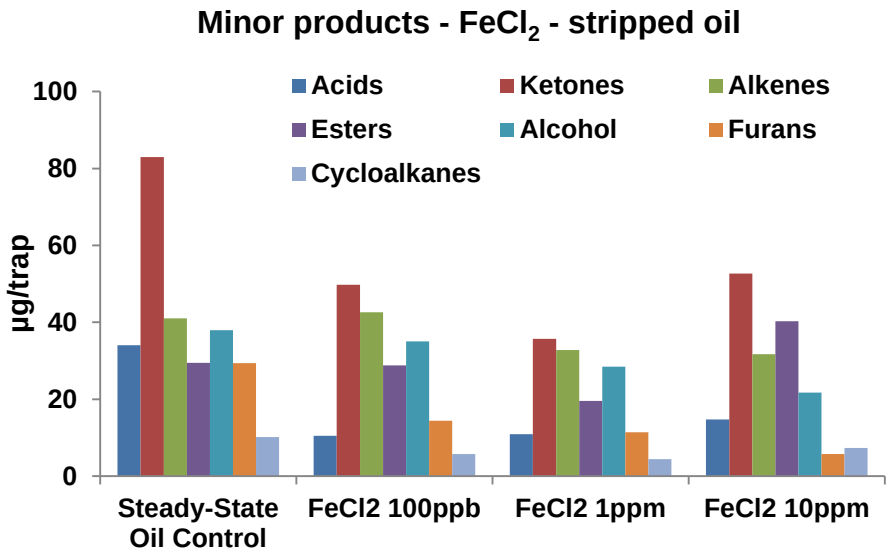


Figure 20. Effects of ferrous iron on levels and distribution of products in a stripped corn oil/HOSO blend heated at 180°C for 3 hours under 2 bars pressure (air).

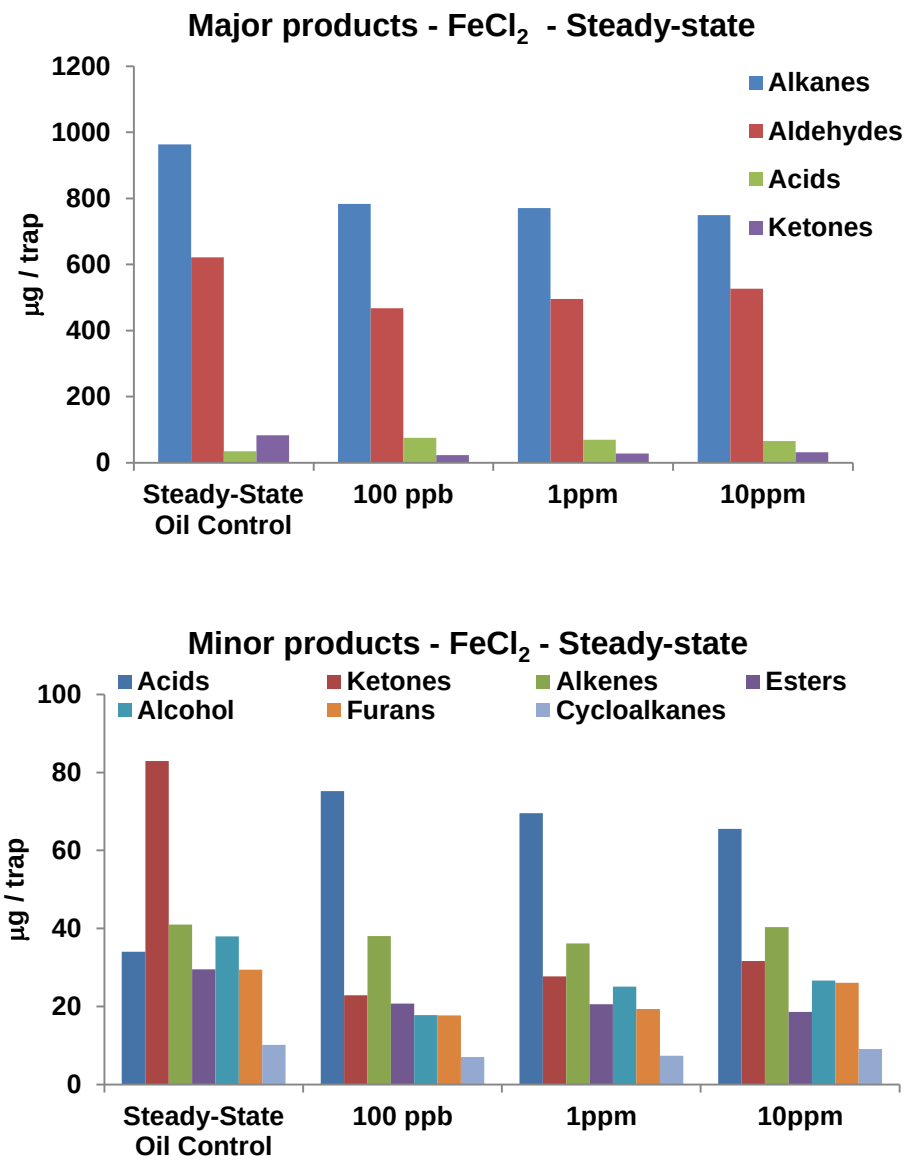


Figure 21. Effects of ferrous iron on levels and distribution of products in a steady-state corn oil/HOSO blend heated at 180°C for 3 hours under 2 bars pressure (air).

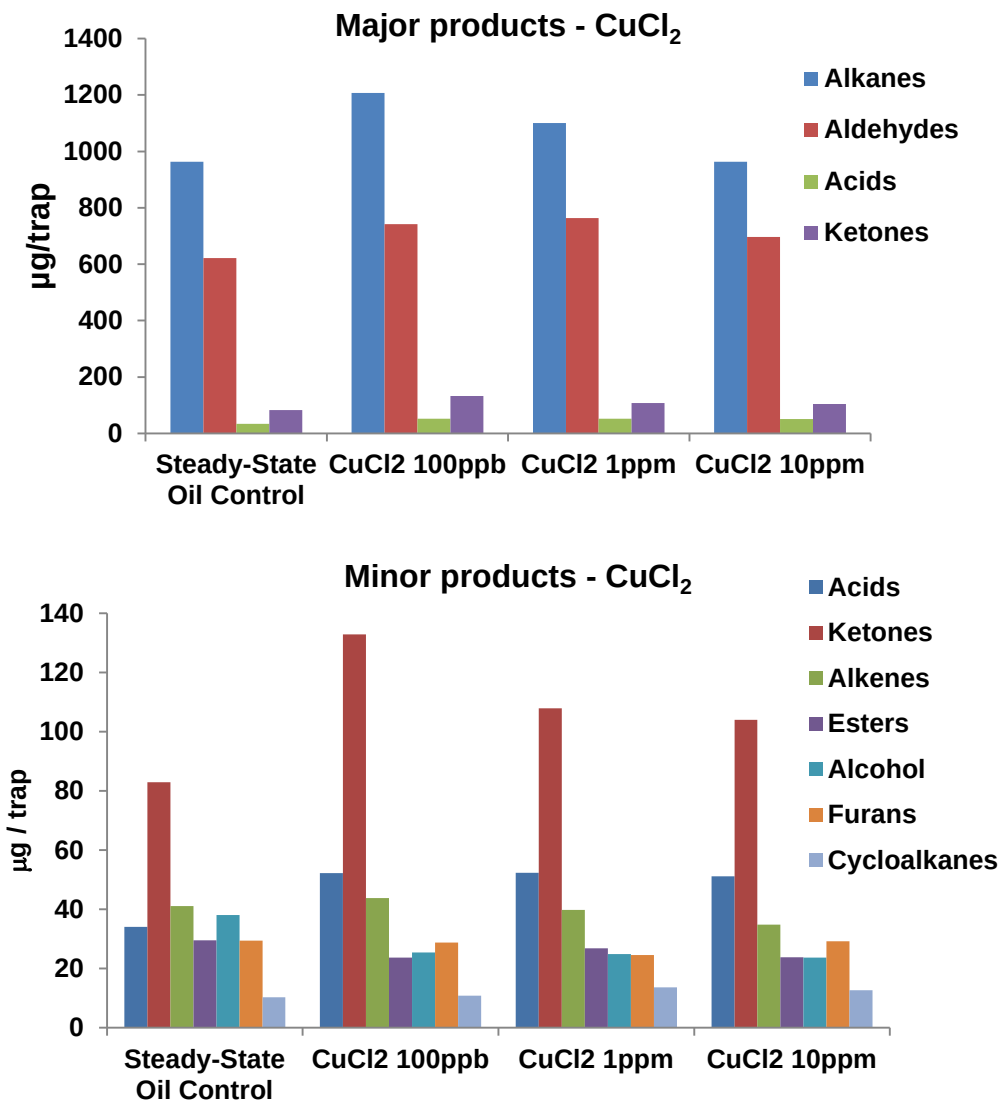
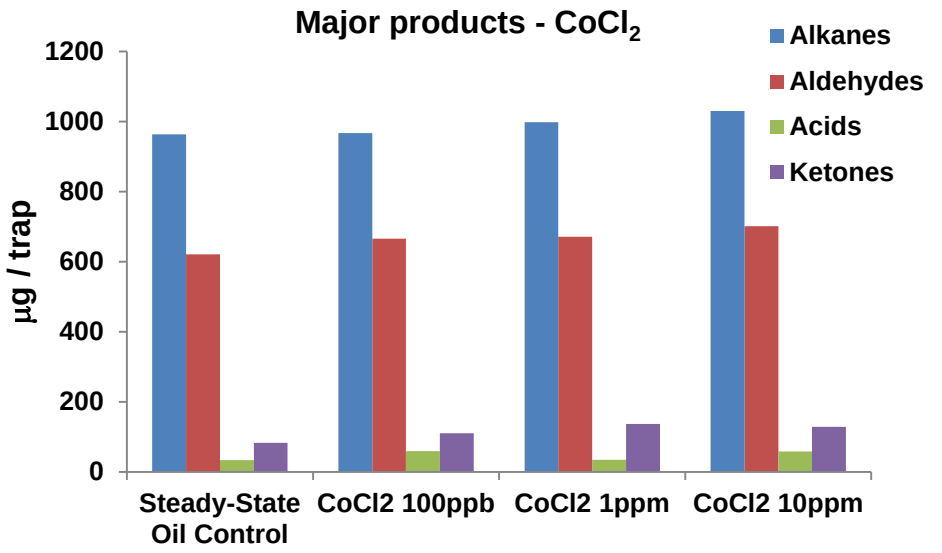


Figure 22. Effects of cupric copper on levels and distribution of products in a steady-state corn oil/HOSO blend heated at 180°C for 3 hours under 2 bars pressure (air).



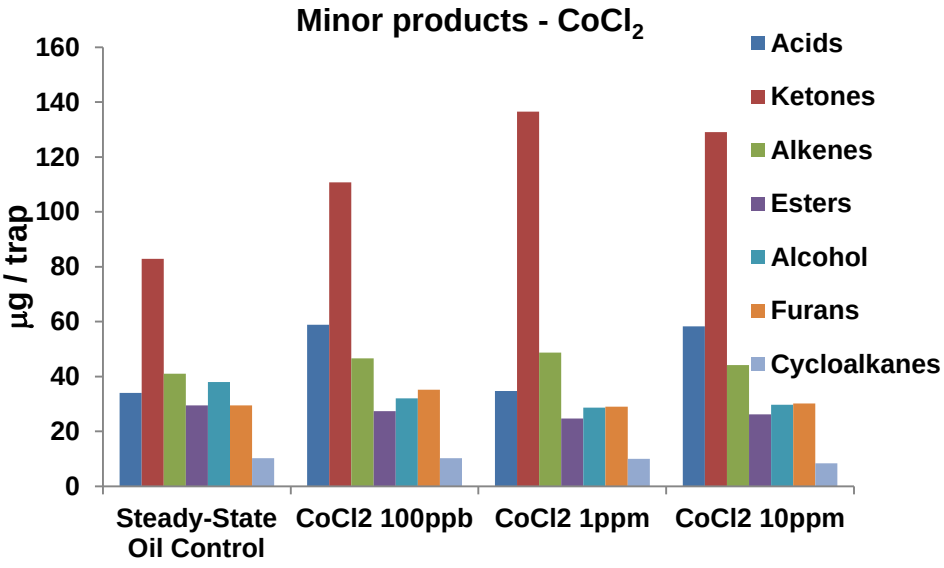


Figure 23. Effects of cobalt on levels and distribution of products in a steady-state corn oil/HOSO blend heated at 180°C for 3 hours under 2 bars pressure (air).

classes of volatiles, and acids increased considerably over controls. Ketones are typical, expected products of metal catalysis in aprotic media, where oxygen insertion to form ketones dominates over electron transfer or form radicals or reduce hydroperoxides (65, 92). In the non-volatile products, LOOH and FFA decreased while aldehydes increased (20), but there was no corresponding alteration of CD.

Thus, the effect of transition metals in frying oils appears to be not in initiating new pathways but in altering secondary degradation mechanisms, diverting existing products into other ones, or in shifting the dominant point of attack to the CH_3 terminus where products are shorter and more volatile. Supporting this proposal are additional observations that transition metals had little effect on fresh oils during heating for three hours (Tian and Schaich, unpublished data). Apparently, metals electron transfer to double bonds cannot compete with thermal scission processes, but oxidation products present more targets for metal interaction.

5.4.4.2 Phospholipids

At 0.02%, phospholipids appear to be strongly antioxidant with dramatic decreases in all volatile products in stripped oil (Table 7, Figures 24 and 25). Both dioleoyl phosphatidyl choline (di-O-PC) and distearoyl phosphatidyl choline (di-St-PC) had similar effects, so the action presumably is related to choline reactivity or physical interactions rather than unsaturation of the fatty acid components. Indeed, antioxidant effects were expected since the positively-

charged choline group has been shown to decompose hydroperoxides by non-radical mechanisms (71). However, if such decomposition occurred, there should have been increased ROH products, but that did not occur. In addition, concentrations of soluble oxidation products were not affected by PC, so any action involving the hydroperoxides can be ruled out. Other mechanisms must be active.

One possibility is physical rather than chemical intervention, where the positively-charged choline binds to volatiles and prevents their release. This action would increase products retained in the oil, but no increases in non-volatile conjugated dienes, hydroperoxides, or aldehydes were observed (20). Alternatively, PC is known to bind water strongly so perhaps it binds traces of adventitious moisture present in the oil. Without additional detailed analyses, at the present time we can only surmise that the PC interacts by an unknown mechanism that by-passes these main products or that PC interacts with a different set of products than those detected in solution assays.

Surprisingly, in steady-state oil (Table 7) and at 0.05% concentrations in stripped oil (Figure 24), PC showed much less antioxidant effect on volatiles. The micromolar concentrations of PC used in both experiments were above the critical micelle concentration for this phospholipid (0.46 nM) (93) so PC micelles are already present. However, new lamellar structures or liquid crystals may form at the higher concentration, preventing access of the choline group to fatty acids

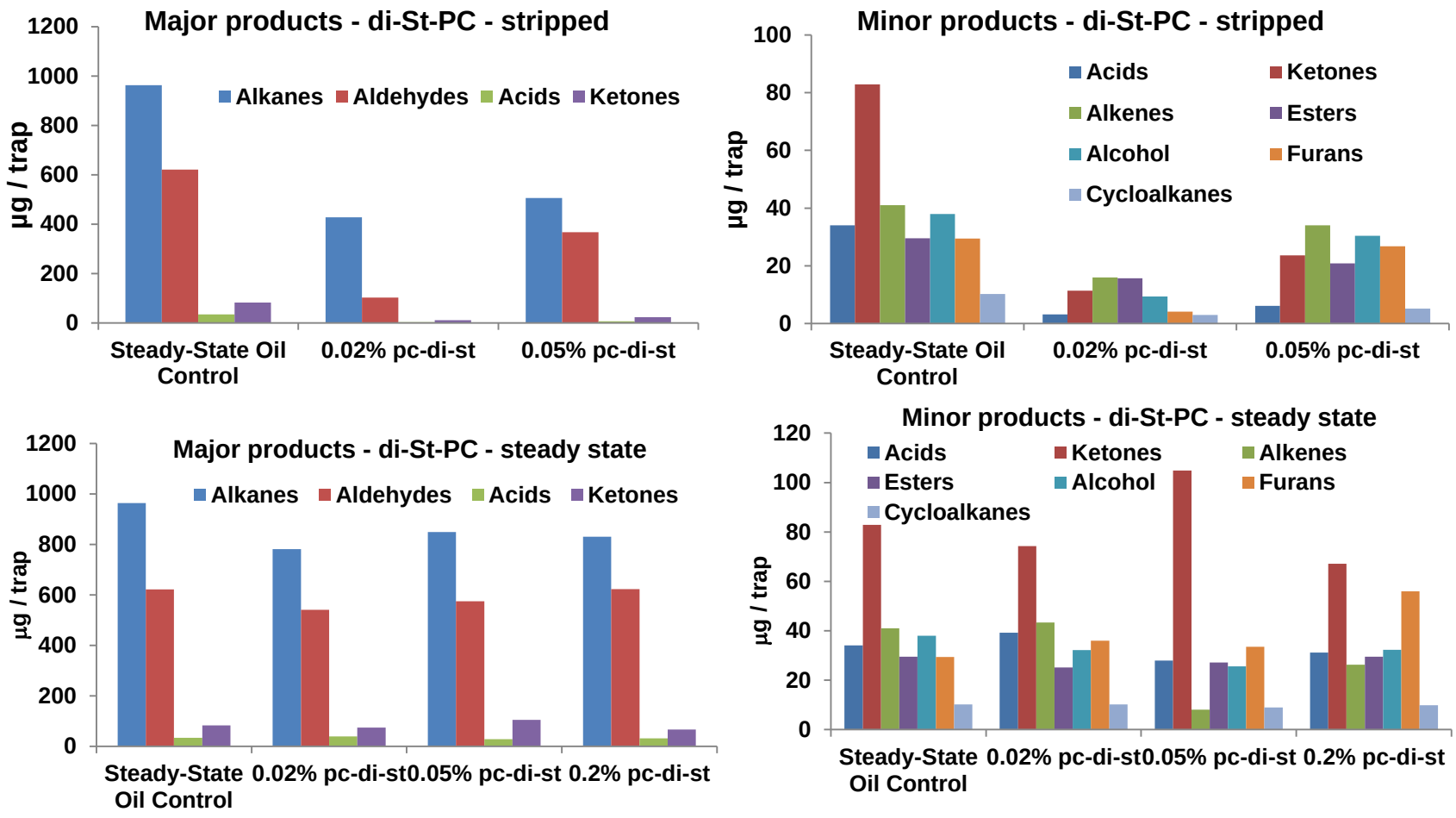


Figure 24. Effects of saturated phosphatidylcholine on degradation product levels and distributions in a stripped (top) and steady-state (bottom) corn oil/HOSO blend heated at 180°C for 3 hours under 2 bars air pressure. Steady-state oil control as a reference.

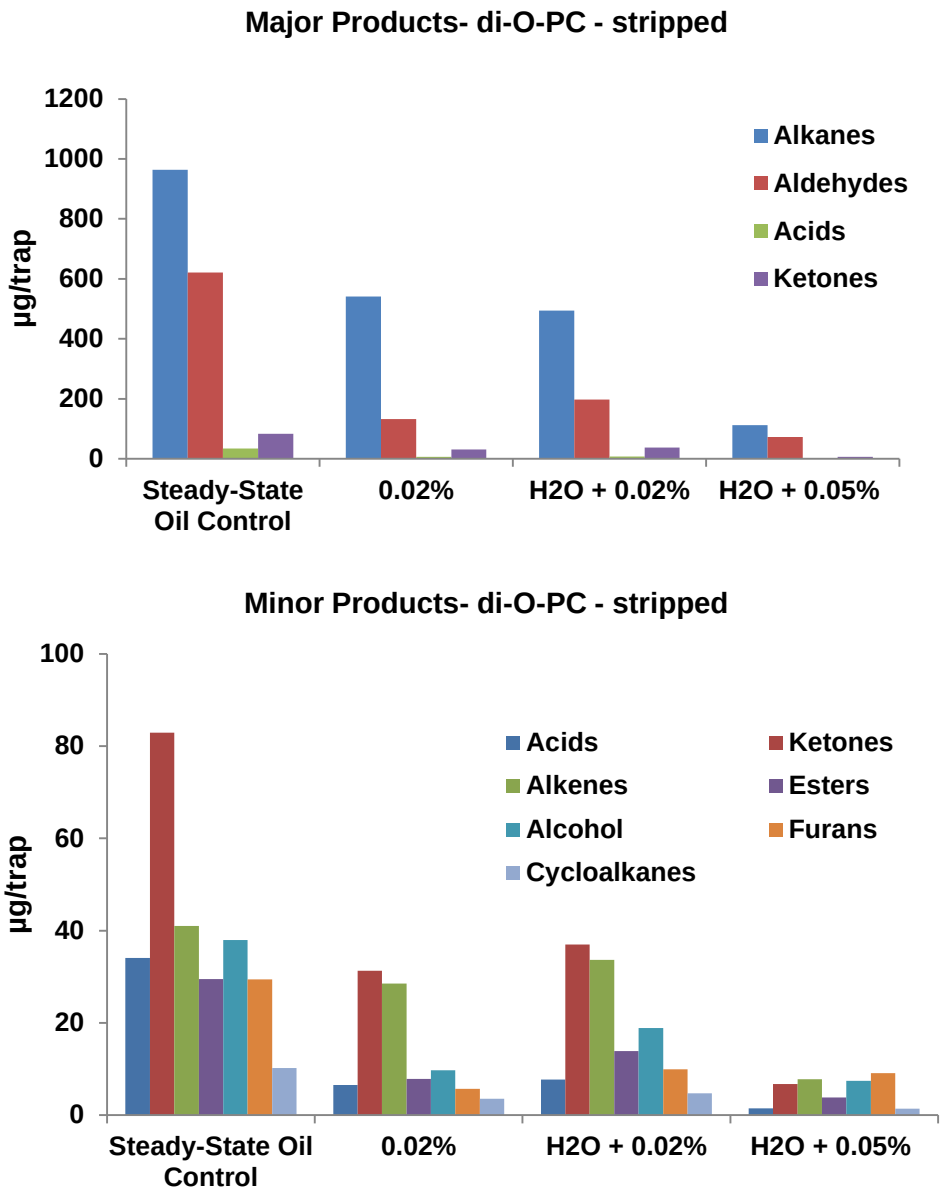


Figure 25. Effects of unsaturated phosphatidylcholine on degradation product levels and distributions in a stripped corn oil/HOSO blend heated at 180°C for 3 hours under 2 bars air pressure. Steady-state oil control used as a reference.

in the triacylglycerols. It is also possible that PC binds some of the oxidation products and decreases their volatility.

5.4.4.3 Free fatty acids

In general, the presence of free fatty acids alone in oils, in the absence of other factors, reduced concentrations of volatile products (Table 6). The proportions of reduction and the distribution of products showed no obvious change with saturation of the fatty acid, so any effects appear to be more related to the acid group than the acyl side chains. At the same time, addition of fatty acids reduced oxygen consumption (oleic acid more than stearic), but did not alter conjugated dienes, hydroperoxides, aldehydes, or free fatty acids. The latter was unexpected since acids have been shown to decompose hydroperoxides by non-radical mechanisms (71).

Graphs of volatile product levels and distributions as a function of fatty acid saturation and concentration (Figures 26 and 27) reveal more clearly the nuances of fatty acid actions. With stearic acid, depression of primary products – alkanes, aldehydes, and ketones -- is greater than secondary products, with most notable reduction in ketones (Figure 26). This may be explained by two actions: 1) hydrogen bonding of the –COOH group to polar oxidation products and physical repression of volatilization, and 2) binding of metals by the –COOH group and limitation of oxygen insertions to form ketones. Metal-binding was most certainly occurring to reroute ketones, but non-volatile products did not change so simple repression of volatilization does not seem likely.

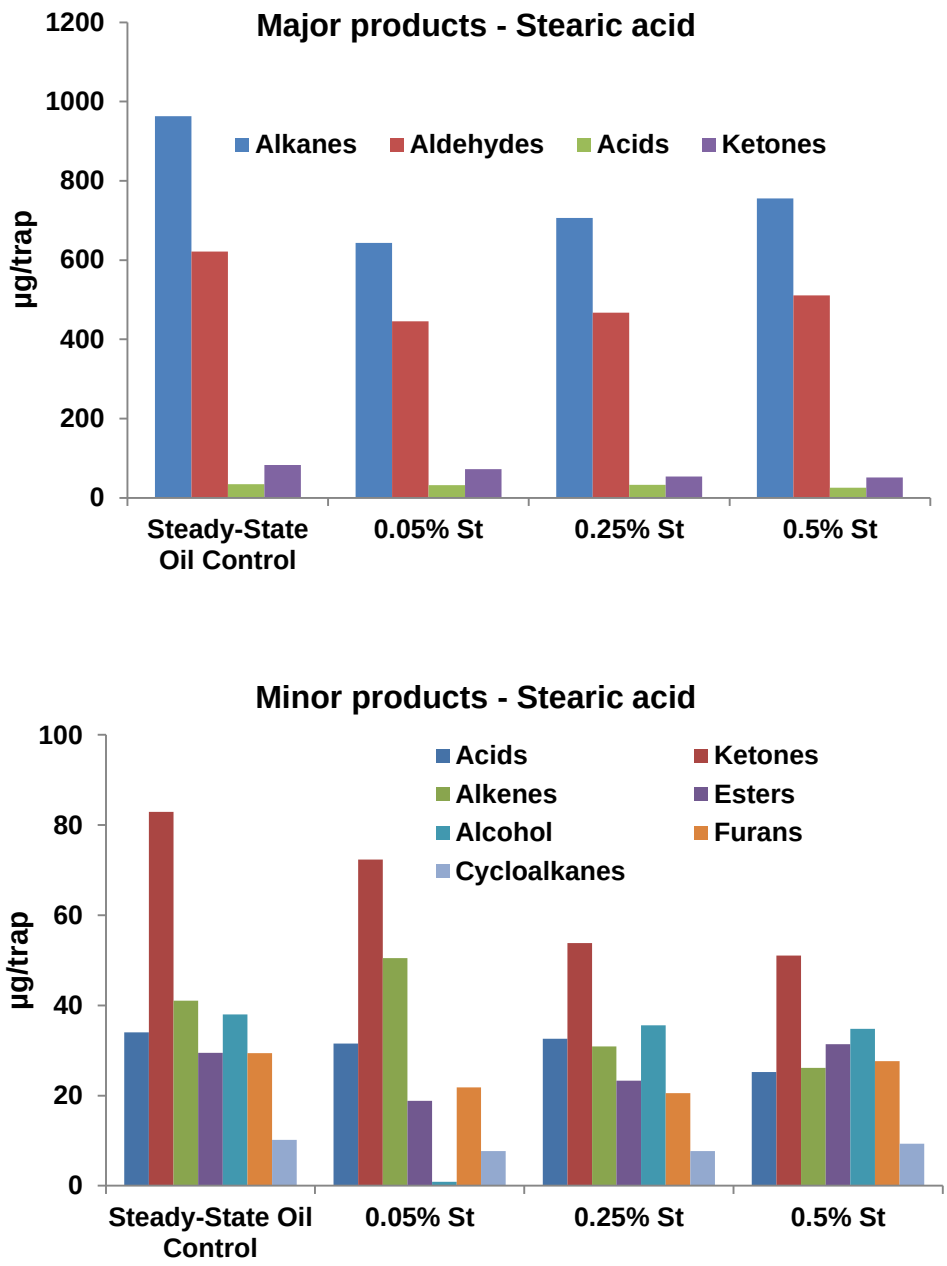
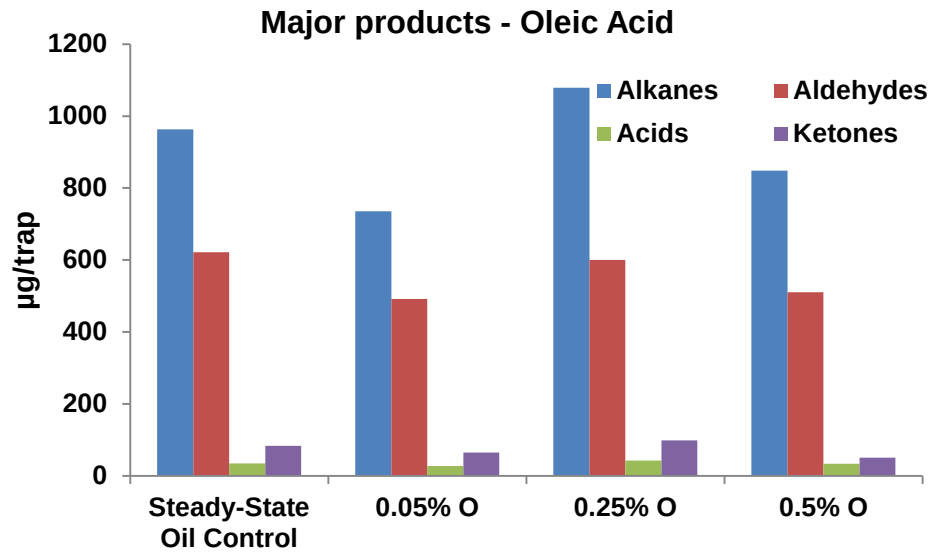


Figure 26. Effects of saturated fatty acids (stearic) on degradation product levels and distributions in a steady-state corn oil/HOSO blend heated at 180°C for 3 hours under 2 bars air pressure.



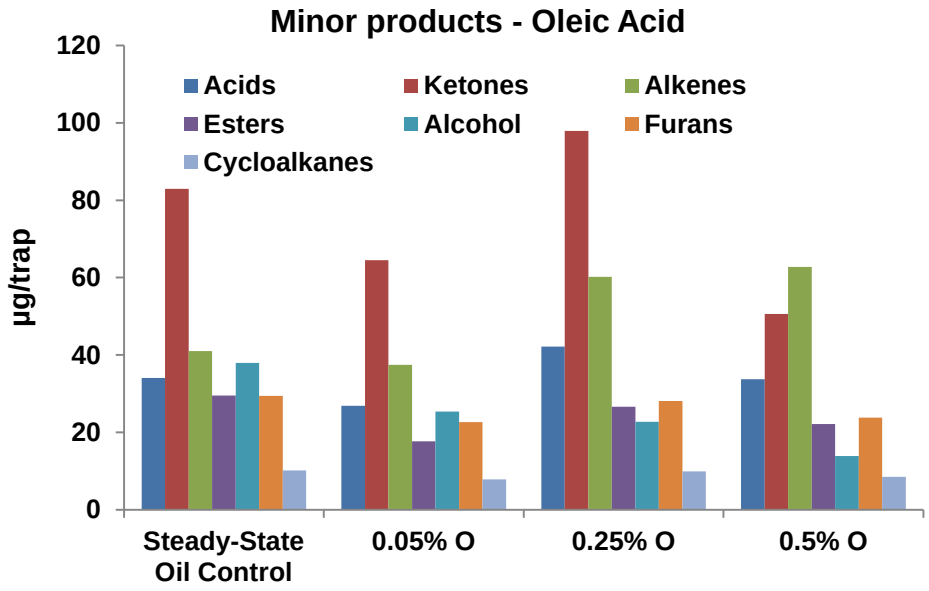


Figure 27. Effects of unsaturated fatty acids (oleic) on degradation product levels and distributions in a steady-state corn oil/HOSO blend heated at 180°C for 3 hours under 2 bars air pressure.

Introduction of a double bond in oleic acid reduced the antioxidant effects of fatty acids and increased detected alkenes. The alkenes may be explained by preferential scissions occurring near the double bond of the oleic acid, as has been noted by Nawar (16). The increase in volatiles relative to stearic acid very likely arises from reactions involving the double bond, but the products involved are not those detected in non-volatile assays.

Unidentified products increase with fatty acids. This observation together with the lack of change in measured non-volatile oxidation products and the double bond actions noted above suggest that fatty acids mediate additional interactions or reactions that fall outside current analyses and understanding. Additional detailed studies will be needed to elucidate all the pathways by which fatty acids act in oils.

5.4.4.4 Water

Water has been reported as both a pro- and anti-oxidant in frying oils, and the same paradox was observed in this study. In our studies, water was the only factor that markedly increased oxygen consumption during heating, yet corresponding changes in non-volatile products were minimal (20) and volatile products decreased dramatically (Table 8). Dana et al (2003) (69) found that 0.7% water injected into oil during frying greatly reduced thermal degradation and formation of secondary products, and attributed the protection to formation of water vapor headspace over the oil and limitation of oxygen access. That

Table 8. Effects of water on volatile lipid degradation products released from a steady-state corn oil/HOSO blend heated at 180°C under 2 bars air pressure for 180 minutes.

			Stripped oil	
	Fresh oil	**Steady-state oil**	**Alone**	**+ 2% Water**
Oxygen consumption	78	104	102	162
Conjug dienes	53	70	58	65
PV	2.7	3.0	2.6	1.86
Aldehydes	135	85	85	76
FFA	0.99	1.22	1.09	1.05
Total volatiles	106	153		
Alkanes	560	963		48
Alkenes	28	41		17
Aldehydes	385	621		70
Acids	17	34		8
Ketones	43	83		12
Esters	16	30		18
Alcohols	38	38		12
Furans	18	29		6
Cycloalkanes	7	10		3
Unknowns	8	6		3

mechanism could be active here since all classes of volatiles decreased dramatically under both inert gases and with added water. However, oxygen limitation would also greatly reduce conjugated dienes, hydroperoxides, and non-volatile aldehydes, which was not observed. Water has been proposed to hydrogen-bond to hydroperoxides, thus preventing their decomposition and enhancement of propagation, but that explanation also is inconsistent with our data. Finally, water is also known to be a radical quencher by H transfer, but then

oxygen consumption would have been repressed, which is opposite what was observed.

As an alternate proposal, we may consider that water decreases local viscosity in the oil and thus facilitates diffusion of oxygen to scission radicals. This would account for the increased oxygen consumption. However, for primary oxidation products to barely change and volatile products to be repressed, water must also catalyze reactions by alternate pathways yielding products that are not volatile and are not detected in the standard product assays. One example may be additions and polymerization which would use up alkanes and prevent formation of peroxides and downstream products that derive from hydroperoxides.

5.4.5 Effects of water-catalytic factor interactions on lipid degradation extent and patterns

Effects of water-factor interactions on oxygen consumption and oxidation products in stripped and steady-state corn oil/HOSO blends are listed in Table 8. Water content in all cases was 2%, and concentrations of metals, phospholipids, and fatty acids varied. Stripped oil was used in some experiments to eliminate protection afforded by 30 ppm citric acid present in fresh and steady-state oils, as well as to reduce levels of tocopherols naturally present in the oils. It was hoped this approach would provide a clearer picture of reactions occurring in the heated oils.

Table 9. Effects of 2% water-catalytic factors interactions on volatile product yields and distributions in steady-state corn oil/HOSO blends heated at 180°C for 3 h under 2 bars air pressure.

	OC[1]	TV[2]	Alkanes	Aldehydes	Acids	Ketones	Alkenes	Esters	Alcohols	Furans	C-alkanes[3]	"X"
SS Control	106.54±0.02		963	621	34	83	41	30	38	29	10	6
2% H20 alone, Stripped	122.80±10.46	239	48	70	8	12	17	18	12	6	3	3
2% H2O plus:												
$FeCl_2$ stripped												
100pppb	143.88±10.98	678	482	118	4	27	23	8	7	6	2	1
1ppm	118.36±5.23	885	605	133	8	13	7	6	48	8	3	3
10ppm	122.06±1.05	835	603	161	7	40	33	11	12	9	3	5
Fe(III)Stearate stripped												
0.1ppm		638	333	188	6	22	29	18	24	10	4	4
1ppm		871	567	182	9	36	29	10	22	10	2	5
$CuCl_2$ steady-state												
100ppb		1125	439	483	40	54	20	15	24	37	7	7
1ppm		1038	402	456	32	47	16	11	25	37	6	6
10ppm		1452	538	642	47	66	25	12	47	55	8	11
Di-St-PC steady state												
0.02%	100.60±9.42	1790	875	656	38	69	41	14	47	34	8	6
0.05%	145.36±3.92	1819	911	652	36	72	49	13	30	39	8	8
0.20%	149.80±1.83	1921	904	723	35	63	39	24	52	56	9	16
di-St- PC Stripped												
0.02%		1099	652	279	7	58	50	12	15	14	5	6
0.05%		326	270	34	1	6	6	2	1	2	1	3

[1] OC= Oxygen Consumption, [2] TV= Total Volatiles, [3] C-alkanes= cycloalkanes

Table 9. 2% water-catalytic factors interactions (continued).

	OC[1]	TV[2]	Alkanes	Aldehydes	Acids	Ketones	Alkenes	Esters	Alcohols	Furans	C-alkanes[3]	"X"
SS Control	106.54±0.02	1850	963	621	34	83	41	30	38	29	10	6
2% H20 alone, Stripped	122.80±10.46	239	48	70	8	12	17	18	12	6	3	3
2% H2O plus: Di-O-PC stripped												
0.02% alone	106.91±0.50	769	541	132	7	31	29	8	10	6	4	2
0.02%	99.49±2.62	823	495	198	8	37	34	14	19	10	5	5
0.05%	99.49±6.80	244	113	73	1	7	8	4	7	9	1	21
Oleic Acid- Steady-state												
0.05%	130.93±2.09	1601	840	558	25	70	43	10	27	19	6	5
0.25%	137.96±7.58	1501	768	525	18	72	40	9	31	28	6	5
0.50%	136.85±3.14	1666	836	594	20	66	31	12	52	36	8	11
Stearic acid- Steady State												
0.05%		1012	386	436	38	53	21	13	31	20	6	9
0.25%		1140	447	483	41	59	25	14	36	26	6	3
0.50%		2121	1014	778	46	70	39	16	80	58	11	8
Stearic acid- Stripped												
0.05%		887	156	191	15	22	53	50	24	8	7	4
0.25%		858	118	143	10	15	52	20	16	8	4	1
H2O + 0.05%		664	76	97	5	10	21	9	14	6	2	1
H2O + 0.25%		672	91	110	5	11	6	10	29	6	3	2
H2O + 0.5%		529	56	74	5	8	3	6	17	5	1	

[a] Units of products: Oxygen consumption in mmols O_2/ mol triacylglycerols, Volatile product concentrations in mg / headspace (from 10 g oil)

[1] OC= Oxygen Consumption, [2] TV= Total Volatiles, [3] C-alkanes= cycloalkanes

5.4.5.1 Water + metals

Water can change the hydration state of metals, provide a local aqueous microenvironment that may change metal reactivity, and provide a medium that supports ionization. In this study, as with water alone, water in combinations with other factors had paradoxical effects, showing clearly that there are many alternate pathways in play. With respect to volatile products, water enhanced the inhibitory effects of $FeCl_2$ (Figure 28) and converted copper from pro- to mildly anti-oxidant (Table 9) (Figure 29). In general effects of metals were most notable in the minor products where production of ketones was reduced while acids were enhanced. In general, inhibition of products increased with metal concentration. In stripped oil, water-$FeCl_2$ acted more strongly in initial reactions generating alkanes and aldehydes, while the reverse occurred in steady-state oil (greater effects on secondary products).

5.4.5.2 Water + phospholipids

The large amounts of water bound by phosphatidylcholine can alter its interactions with metals and enhance its activity as a nucleophile.

In stripped oil, distearoyl phosphatidylcholine erased the enhancement of oxygen consumption that water had induced and levels of all products were greatly reduced (Table 9, Figure 30); 0.05% di-St-PC + water nearly eliminated secondary volatile products. In contrast, water and di-St-PC together in steady-state oil (Figure 30) increased oxygen consumption over water alone (effects were synergistic, not additive), and nearly all products remained at control levels. This is to say, in steady-state oil, both catalytic and protective effects of water

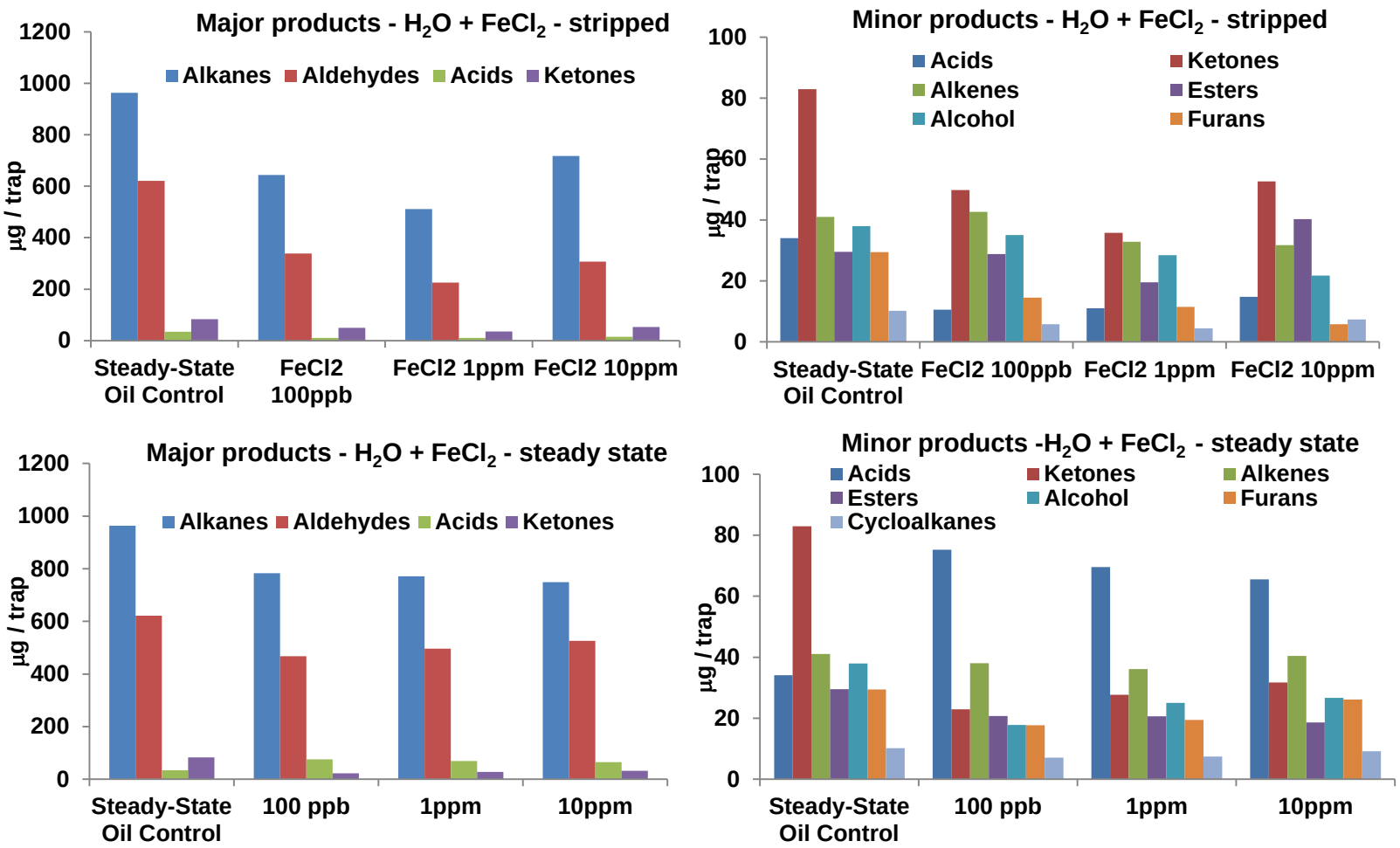


Figure 28. Effects of water-$FeCl_2$ interactions on generation of volatile degradation products from stripped (top) and steady-state (bottom) corn oil/HOSO blends heated at 180°C for 3 hours under 2 bars air pressure.

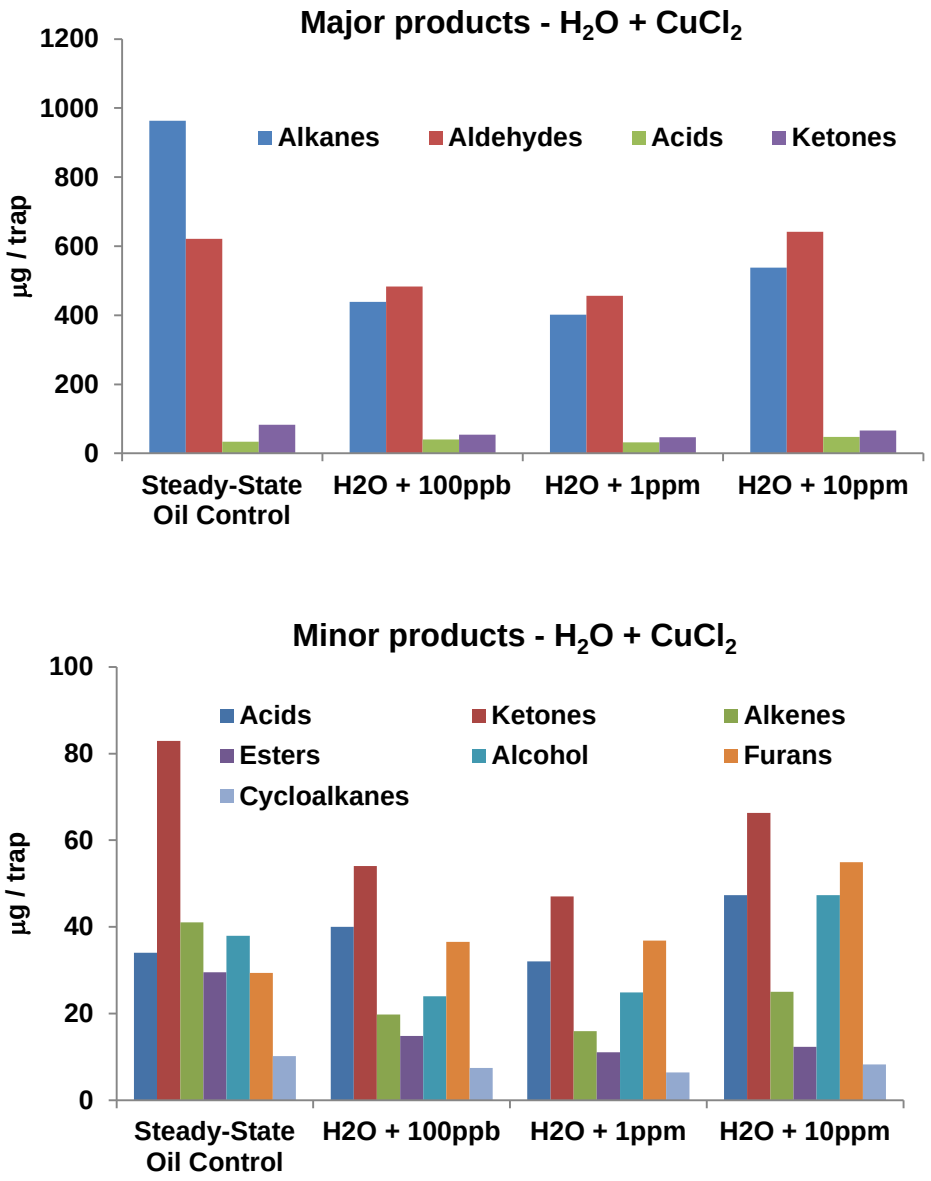


Figure 29. Effects of water-cupric copper interactions on generation of volatile degradation products from a steady-state corn oil/HOSO blend heated at 180°C for 3 hours under 2 bars air pressure.

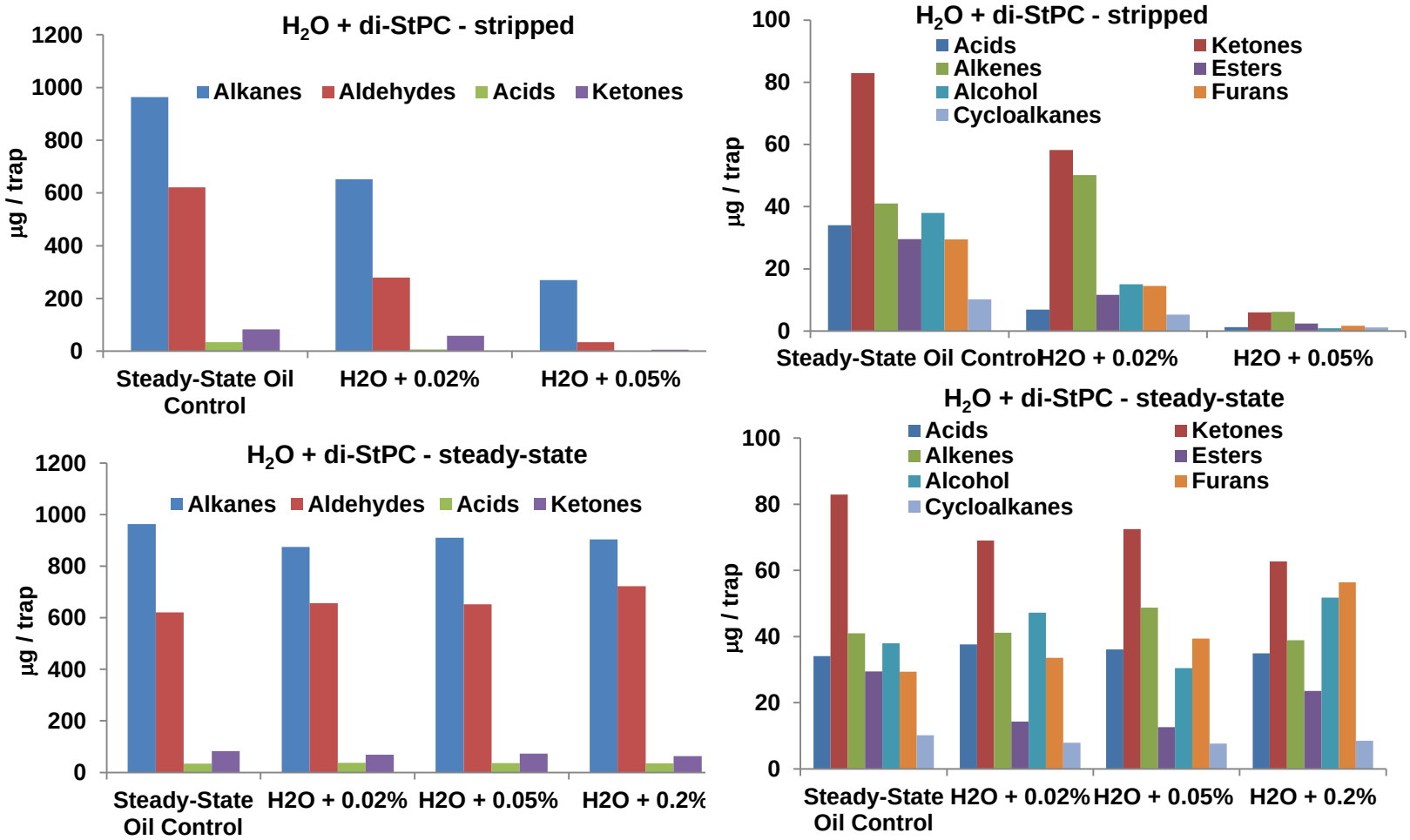


Figure 30. Effects of water-phosphatidylcholine interactions on generation of volatile degradation products from stripped (top) and steady-state (bottom) corn oil/HOSO blends heated at 180°C for 3 hours under 2 bars air pressure.

and di-St-PC were nullified. Evidently, some component in steady-state oil blocked the active sites of di-St-PC and interfered with the actions of water. These observations once again indicate that oil chemistry changes as frying time and thermal degradation increase, and have significant practical implications for stabilization during industrial frying.

5.4.5.3 Water + fatty acids

As with metals, water can change the hydration state of the –COOH group, support its ionization, and alter its interactions with other molecules. Water also catalyzes formation of complex aggregates between metals, fatty acids, and polar degradation products, particularly aldehydes. The acid group forms hydrogen-bonded dimers and micelles, binds metals to form carboxylates that increase solubility of metals in oils (94) and are also catalytically-active (95). It has been generally accepted that introduction of free fatty acids destabilizes frying oils, but consideration of the multiple pathways open to fatty acids makes their actions in oils difficult to predict.

In steady state oil, stearic and oleic fatty acids slightly decreased oxygen consumption and volatiles (Table 9). Addition of water with both fatty acids greatly increased oxygen consumption while decreasing hydroperoxides and volatile oxidation products more than by acids alone (Table 9, Figure 31). This is an enhancement of the acid action since water alone nearly eliminates volatile products. Interestingly, ketone products do not change with acid concentration, indicating that metal binding is probably not involved. Also, effects of acids

appear to be biphasic and concentration-dependent. Antioxidant effects are relatively constant at acid concentrations of 0.25% and lower, but at 0.5% acids nearly all volatile products increase and the acids appear to become pro-oxidant.

The situation changes in stripped oil (Figure 32). Stearic acid alone markedly reduced both major and minor volatile products, and addition of water reduced volatiles still further. In this case, alkanes and aldehydes slightly increased with acid concentration (but still remained depressed), while secondary volatiles decreased continuously with acid concentration, even at 0.5%.

The differences between the two oils suggest that all the acid actions described above probably are present and change in dominance depending on conditions. In stripped oil, metals and the citric acid normally added to complex metals have been removed, along with hydroperoxides that can hydrogen-bond to acid groups and aldehydes that can complex to metal soaps (carboxylates). Thus, the acid group is free to act directly, e.g. by decomposing hydroperoxides. However, metals, citric acid, hydroperoxides, and aldehydes are all present in steady-state oil. Formation of metal complexes converts acids to potentially catalytic carboxylates, and the presence of pre-formed aldehydes allows formation of catalytic aggregates. Addition of water facilitates ionization of the acid, which in turn should also facilitate carboxylate formation and perhaps other actions as well.

To further investigate metal- fatty acid – water interactions, 0.05% oleic acid (lowest concentration) and varying concentrations of $FeCl_2$ were added to oils with and without added water in steady-state oil. Whereas oleic acid and

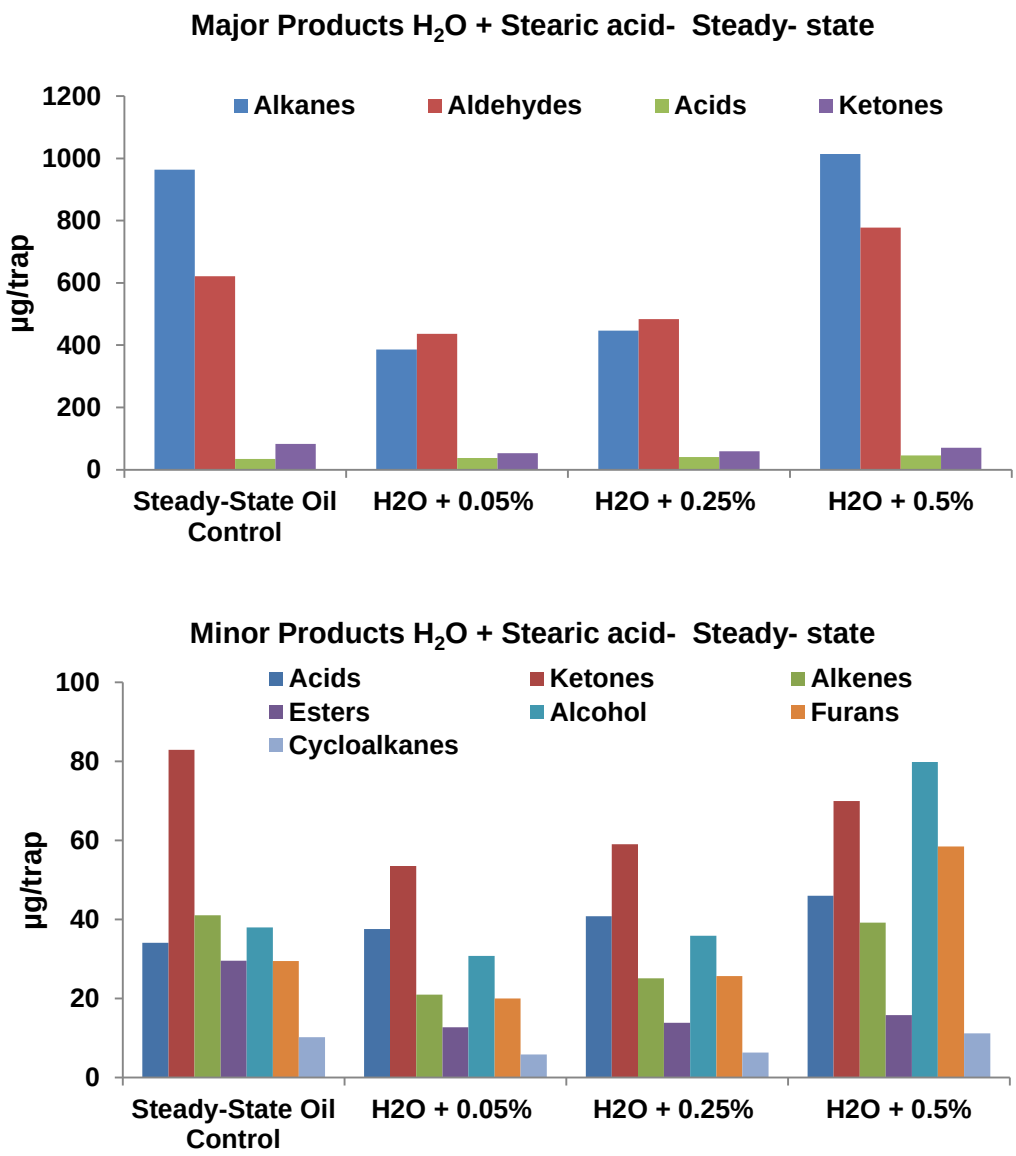
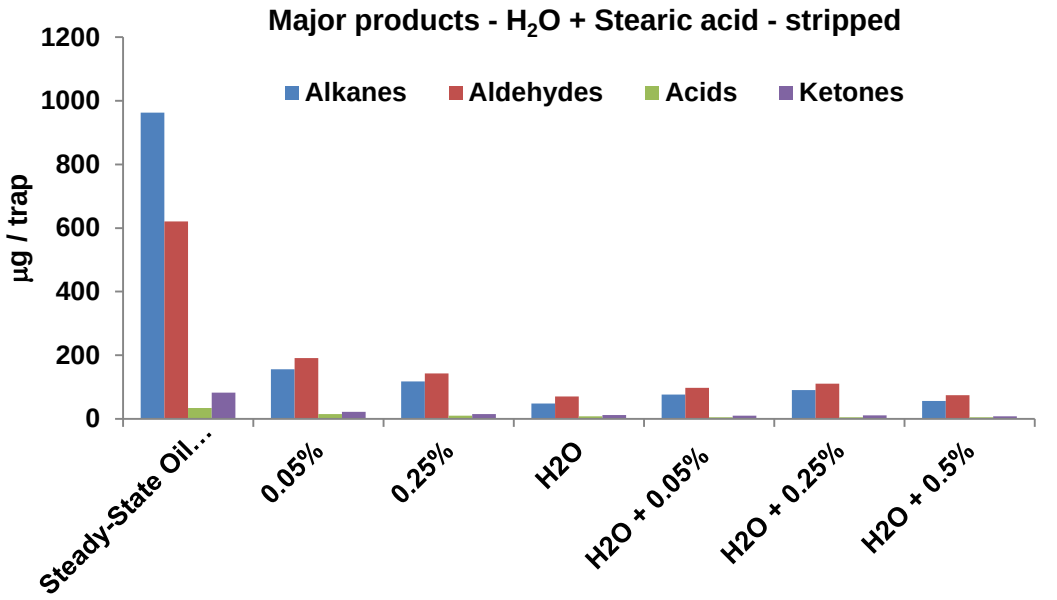


Figure 31. Effects of water-stearic acid interactions on generation of volatile degradation products from steady-state corn oil/HOSO blends heated at 180°C for 3 hours under 2 bars air pressure.



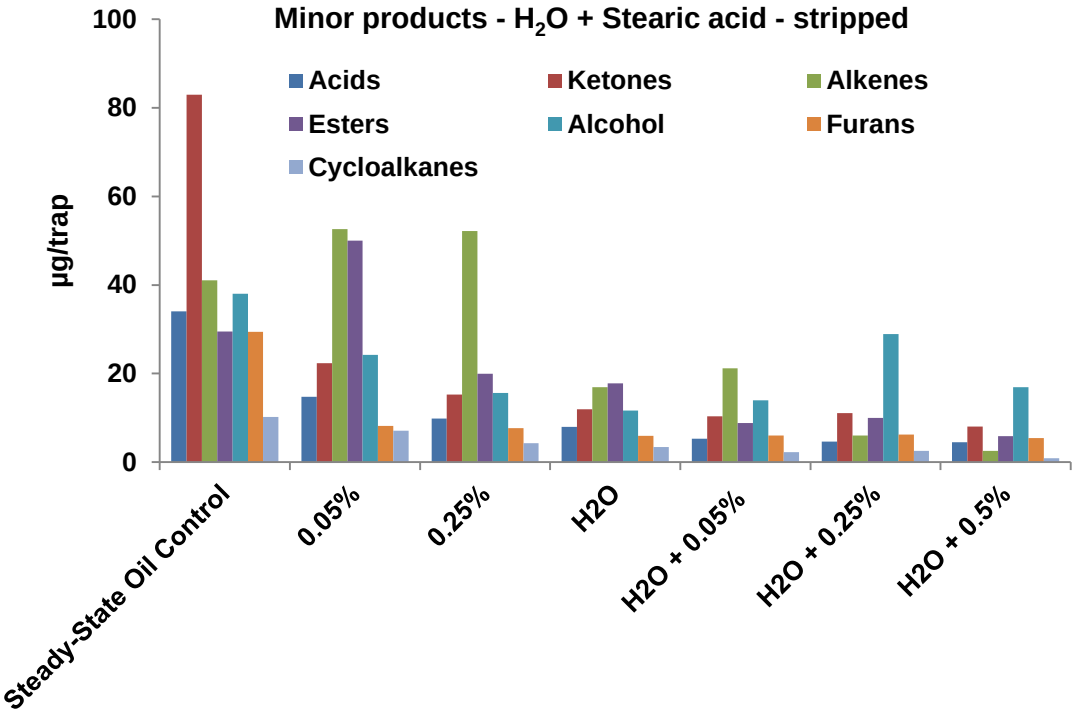
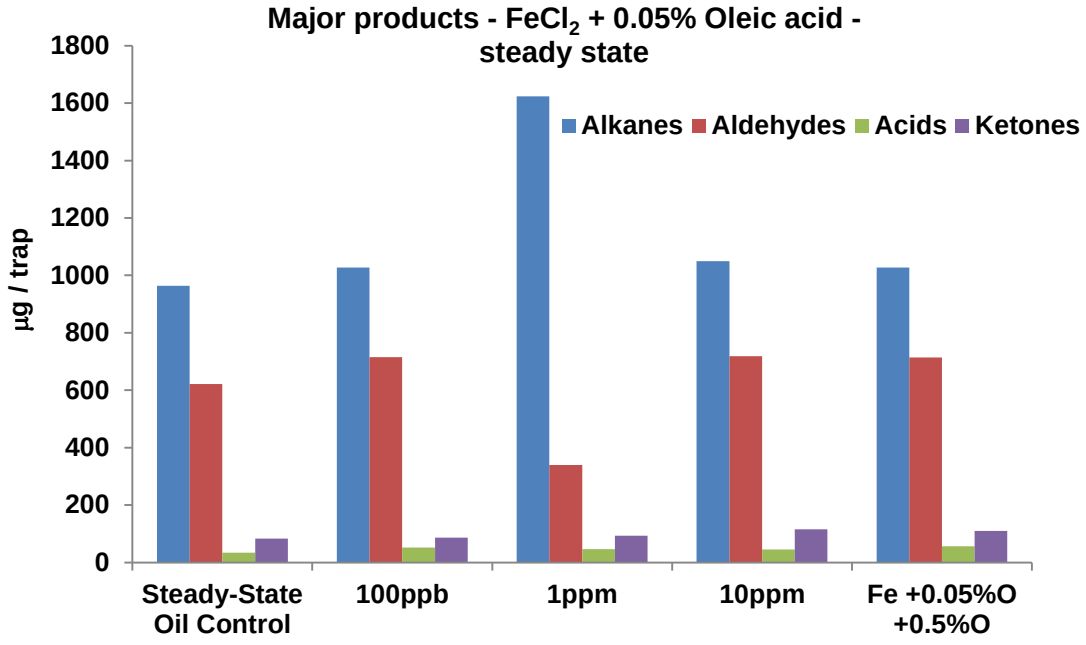


Figure 32. Effects of water-stearic acid interactions on generation of volatile degradation products from stripped corn oil/HOSO blends heated at 180°C for 3 hours under 2 bars air pressure.

$FeCl_2$ were each mildly antioxidant alone, inhibition of oxygen consumption and non-volatile oxidation products increased when these two factors were together (20), but at the same time there was little effect on volatiles (Figure 33). The one exception was increasing ketones which reflected the metal actions.

When water was added to this mix, oxygen consumption increased markedly, but now oxidation products and also major volatiles (alkanes, aldehydes, and ketones) decreased with iron concentration (Figure 34). Secondary volatiles were not affected much.

Why water should so dramatically increase oxygen consumption yet inhibit generation of standard lipid oxidation products remains an enigma. Nevertheless, it seems clear from results that water and fatty acid-metal interactions play very important roles in modifying degradation chemistry in stated oils, more detailed studies of the chemistry involved are needed to elucidate the anti- and pro-oxidant mechanisms involved.



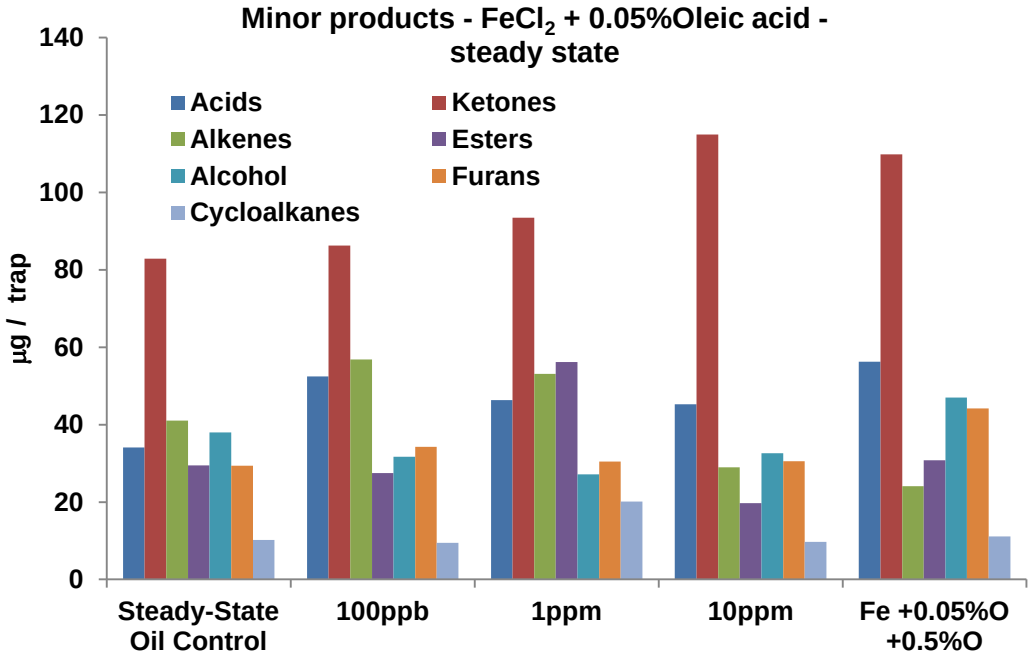


Figure 33. Effects of $FeCl_2$-oleic acid interactions on generation of volatile degradation products from steady-state corn oil/HOSO blends heated at 180°C for 3 hours under 2 bars air pressure.

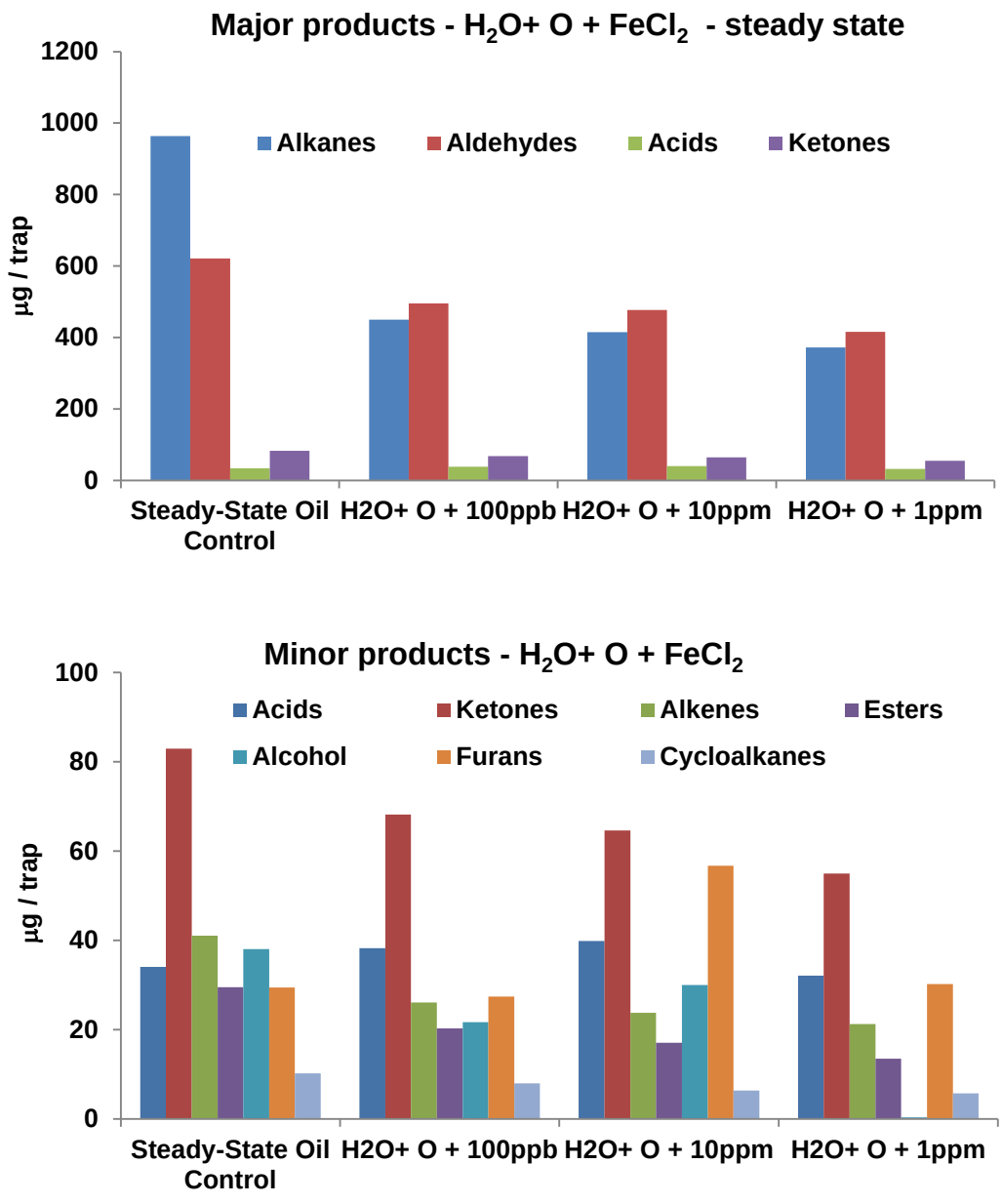


Figure 34. Effect of three-way water-oleic acid-$FeCl_2$ interactions on generation of volatile degradation products in a steady-state corn oil/HOSO blend heated at 180°C for 3 hours under 2 bars air pressure.

5.5 Objective 2b. What are relative contributions of thermal scission from autoxidation reactions?

We recognize that measuring volatiles can skew views of chemical reactions somewhat since only volatile and hence shorter chain products are detected. At the same time, heating oil in a closed system offers unique opportunities to collect products that are lost when frying studies or operations are conducted in the open. Overall, both the patterns of products and relative kinetics of product formation support thermal scission as the dominant process during the three hours of heating in this study.

Volatile compounds with up to 13 carbons were detected. In general, the concentrations of compounds in the different classes of products were highest among the short chains and decreased with chain length. As an example of this pattern, concentrations of carboxylic acids as a function of heating time are shown in Figure 35. These acids are produced either by scissions on the acid end of fatty acid chains together with scission of the ester bond, and possibly also oxidation of aldehydes. Butryic acid, the dominant acid by more than ten times all others. Hexanoic is next, and then formic acid, after that, acid concentrations decrease in order of chain length, with nonanoic acid being lowest. All of these acids are strictly thermal scission products and do not occur during autoxidation of triacylglycerols.

Similarly, short chain volatiles were formed from scissions on the other end of the fatty acids. Pentane was the major alkane, formed from scission α to

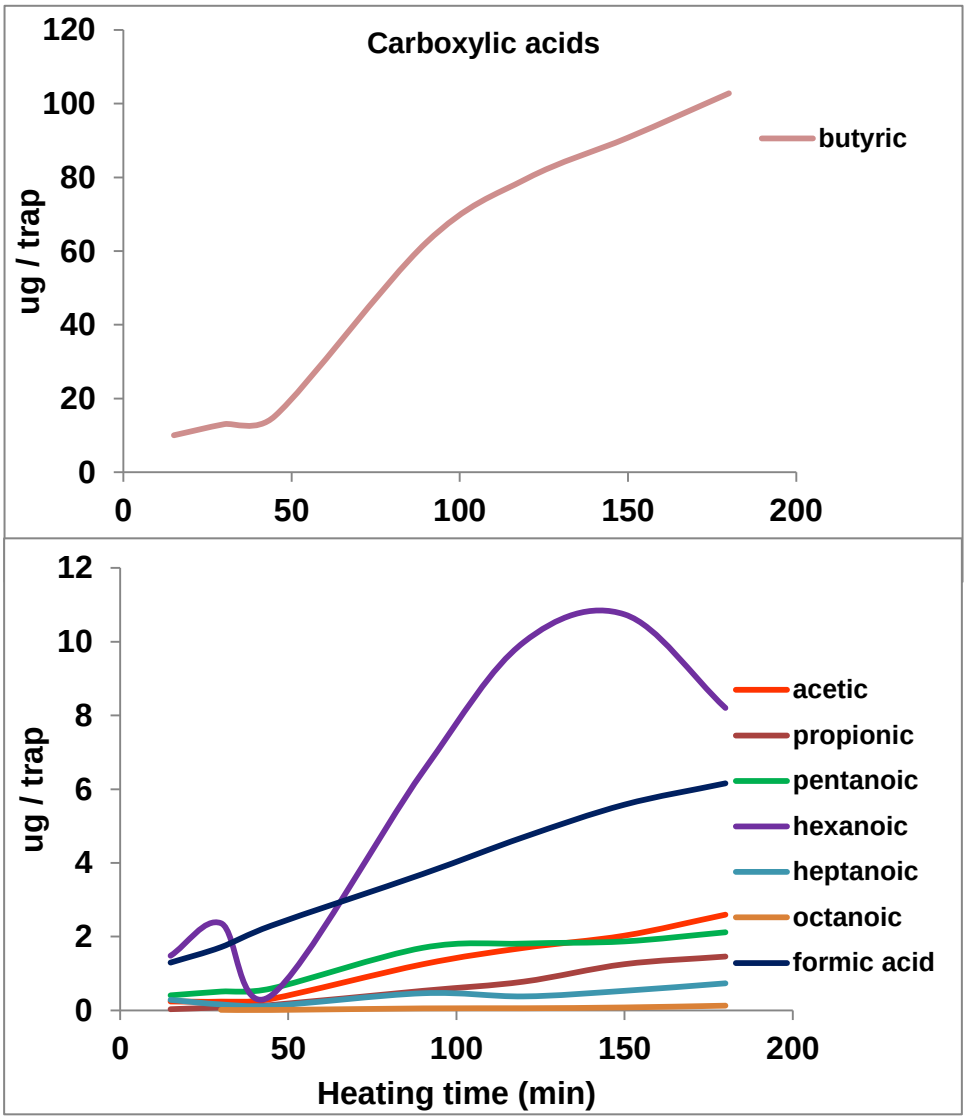


Figure 35. Generation of carboxylic acids during heating of fresh corn oil/HOSO blend at 180°C for 3 hours under 2 bars air pressure.

the C12-13 double bond of linolenic acid. Pentane can be generated by heat (thermal scissions next to the double bond) or autoxidation (scission next to C13 alkoxyl radical), but rapid formation before evolution of other product classes supports thermal scission as the source. The same pattern was followed with aldehydes, ketones, alcohols, and alkenes. This loading of products with short chain components, especially less than six carbons, is not typical of autoxidation,

but is certainly consistent with the process described by Nawar in which scission occurs at all points along acyl chains, with preference to scissions 1, 2, and 3 carbons removed from the ester group and double bonds.

Kinetics of product generation also supports thermal scission as the initiating process for overall degradation. In particular, alkanes are produced before and at much higher levels than conjugated dienes, which only appear after hydrogens are abstracted from polyunsaturated fatty acids.

Hydroperoxides begin to form as soon as the temperature begins to rise in the oil. It can be argued that this indicates autoxidation, but solution assays do not distinguish types of hydroperoxides or the fatty acids where they are formed. Because alkanes are generated almost as fast, and hydroperoxide generation occurs for some time without accompanying conjugated dienes, sources of hydroperoxides other than from H abstraction from allylic carbons (autoxidation) must be present. Our data is consistent with addition of oxygen to thermal scission radicals and subsequent H abstraction to form terminal hydroperoxides, which can then decompose to aldehydes or rearrange to other products.

5.6 Data demonstrating participation of autoxidation reactions

Obviously, the formation of conjugated dienes indicates that hydrogen abstraction and initiation of autoxidation chains occurs during heating. However, hydroperoxides and alkanes form faster, which indicates that hydrogen abstraction to generate the conjugated dienes is a secondary process.

Products expected from scission of the alkoxyl radicals formed during autoxidation i.e. at C9 and C13 of linolenic acid and at C8-C11 in oleic acid, were either not observed or were minor products in the current study, with the exception of hexanal. Hexanal was a major aldehyde, although it could not be quantitated with certainty due to co-elution with octane and butyric acid. Hexanal can be generated by at least three mechanisms: 1) α-scission of alkoxyl radicals at C13 on linolenic acid, 2) secondary oxidation and decomposition of 2,4-decadienal, and 3) scission between C12 and C13 of oleic acid or any medium to long chain saturated fatty acids, followed by oxidation. Hence, the presence of hexanal itself is not diagnostic of any specific mechanisms.

Decadienal, another product expected from lipid oxidation and indeed considered to be a major product in frying oils, was not found in this study. Decadienal is volatile so should have been detected in the volatiles. It is possible that it decomposed to hexanal and octane, or other products, but that seems unlikely considering its common appearance in other studies. Studies are in progress to determine the stability and volatility of decadienal when heated in the Oxipres.

2-Pentyl furan, another typical autoxidation product formed by internal addition of a C13-$O^{\bullet}$ to the C9-C10 double bond of linoleic acid, was detected as a minor product present at very low concentrations.

The fact that removal of oxygen shuts down all generation of all products is suggestive of autoxidation involvement.

Summary and Conclusion:

Comparisons of oxygen consumption, non-volatile primary oxidation products, and volatile products provide useful insights into thermal degradation processes that would not be available from any one of these methods alone. Mismatches among results and non-constant shifts among products show clearly that thermal degradation of lipids is a very complex process that involves many reactions, not all of which could be accounted for by the data from this thesis and its partner, the Tian dissertation.

Results from this integrated project support thermal scissions as the primary driving force underlying thermal degradation of lipids. Autoxidation occurs secondary to and is initiated by thermal scissions. Homologous series of short chain products that would normally be lost by evaporation were trapped and detected in the oxygen bomb. Alkanes and alkenes not found in autoxidation were major volatile products that began accumulating immediately upon heating and preceded development of conjugated dienes. These were formed by thermal scissions occurring at multiple positions along acyl chains. The products identified are consistent with the scission points demonstrated by Nawar's work. The scission fragments can anneal to form alkanes and alkenes, add O_2 to form terminal peroxyl radicals, add $O^{\bullet}$ to form aldehydes directly, or dimerize by addition to double bonds or recombination with other radicals. Peroxyl radicals formed in this way can abstract hydrogens from unsaturated fatty acids to initiate autoxidation chains that amplify thermal scission processes.

Aldehydes were the next volatile products in both timing and quantity of formation. The short chain aldehydes detected were not those typically generated by decomposition of mid-chain hydroperoxides formed specifically in autoxidation. Non-volatile hydroperoxides began forming immediately with heating but were not accompanied by formation of conjugated dienes. We propose that these initial hydroperoxides were terminal hydroperoxides formed by oxygen addition to thermal scission radicals, and that decomposition of these hydroperoxides generated a large of the early aldehydes detected.

Short chain carboxylic acids inconsistent with hydrolysis of fatty acids from triacylglycerols began to develop after decomposition of hydroperoxides and aldehydes, and required oxygen for formation. These acids are most likely oxidation products of thermal scission aldehydes.

Factors usually considered to catalyze lipid oxidation at ambient temperatures by initiating radical chains and decomposing hydroperoxides to enhance propagation rates cannot compete with thermal scissions as initiators in heated oils. Instead, they appear to act on secondary products and alter product decomposition pathways. One notable pattern was a shift in reaction mechanisms for metals in oils, where hydroperoxide formation and decomposition was replaced by oxygen insertion to form ketones.

Reactions and interactions during thermal degradation of oils are complex and it is clear from results of this study that not all pathways and products are being accounted for. More detailed studies including analysis of dimers and other soluble products will be necessary to fully elucidate thermal degradation.

Future Work:

The determination of individual volatile products generated during thermal degradation of a corn oil/HOSO blend has contributed significantly to sorting out some reactions, but other reactions remain unidentified. More data was collected than that presented in this thesis, and analysis of this data must be completed to learn more. In particular, the kinetics of development and decomposition of individual products should help identify preferred thermal scission sites.

This study needs to be repeated with more variations to establish statistical validity and to investigate more fully how chemical and physical factors affect product pathways. Any volatiles analyses must be accompanied by parallel analyses of detailed products remaining in the oil the help identify missing pathways and products, giving particular attention to identifying structures of dimers and polymers. Obviously, such analyses are difficult to perform, but advances in LC-MS and increased availability of Maldi-TOF MS should make the studies feasible. In situ EPR studies of the radicals formed during heating will also help document types of radicals formed and clearly establish the occurrence of thermal scissions.

The failure to detect 2,4-decadienal, thought to be a major thermal autoxidation product, was an enigma for this study. Experiments are underway to track thermal degradation of this product during heating. It also appeared that initial reactions occurred preferentially near the terminal end of acyl chains during the short heating times of this study. A study is needed to determine whether

decadienal and other autoxidation products from the acid end of acyl chains accumulate only after long-term frying and extensive initial degradation.

Results of this study combined with Tian's showed that the catalytic factor perhaps exerting the greatest influence on thermal degradation of oil is water, and that water effects are quite complex and paradoxical. Frying always occurs with food, so water must be considered as a critical determinant of practical frying consequences. Thus, more detailed studies of water effects on mechanisms as well as effects of water levels on degradation kinetics and pathways are clearly needed.

Finally, the large amount of data and numbers of products detected in a study of this type are very difficult to assimilate and integrate manually. Application of new data mining techniques should be quite useful for deriving maximum information from data generated. Chemometrics analysis has been applied to sorting contributions of volatiles to flavors (96). Discussions with the authors suggest chemometrics approaches should be very promising for gleaning important reaction and product patterns from thermal degradation and lipid autoxidation data.

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